SPATIAL LEARNING AND MAP ORIENTATION IN THE RAT: AN INVESTIGATION OF THE ROLE OF POINT OF ENTRY, VISUAL CUES, AND PATH INTEGRATION, USING BEHAVIOURAL PROBES AND PLACE CELL RECORDINGS

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by

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A thesis submitted to the School of Graduate Studies

in partial fulfillment of the requirements for the degree of

Master of Science, Behavioural Neuroscience

Memorial University of Newfoundland

September, 1998

St. John's Newfoundland

Abstract

The purpose of this study was to investigate whether a stable point of entry (POE) is required for rats to solve a spatial learning problem in a curtain-enclosed environment containing a large visual cue panel, and to use both behavioural measures and place cell recordings to determine the basis of these solutions. Ten of sixteen male Long Evans rats learned to locate the baited corner of a square box, regardless of whether they had a fixed or randomly varying POE through the curtain, suggesting that a fixed POE is not necessary for learning on this type of dry-land appetitive task. Probe trials revealed that the rats could use either their internal sense of direction or the visual cue to locate the goal, but rarely used the cue unless explicit measures were taken to disorient them. Disorientation only led to cue use if the animals were also deprived of access to a view of the room prior to passage through the curtain, suggesting that the rats' orientation was being obtained from room geometry. The rats did not seem to be calling up new maps with an orientation anchored to the POE. Place fields maintained stable positions across trials in the box, unless cue rotation was combined with disorientation, in which case fields rotated by an amount corresponding with the rotation of the cue. These results provide converging lines of evidence that rats prefer to use their internal sense of direction in this type of problem, but are willing to rely on visual cues if they have been perceptibly disoriented.

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Acknowledgments

I would sincerely like to thank my supervisors, Drs. Carolyn Harley and Gerard Martin, for their time, patience, and expertise, without which completion of this project would have been impossible. I would also like to thank Dr. Brian Craske for his friendly advice, Dr. John Evans for recommendations on statistical matters, Avery Earle for assistance with computer programming, and Drs. Jeffrey Taube and Bruce McNaughton for contributing helpful comments regarding the difficult matter of obtaining single cell recordings from awake, moving animals.

A special thank-you also to Christina Thorpe, Jeremy Barry, Sonya Cull, and Dave Jones, who were kind enough to lend their hands to the many tasks required to put this project together.

This research was supported by NSERC grants to Drs. Carolyn Harley and Gerard Martin.

1. Introduction

1.1 The purpose of this study

Recent spatial learning experiments have uncovered a surprising dichotomy in the results of disorientation on spatial learning in aversive water maze tasks versus appetitive dry land tasks. Dudchenko et al. (1997) and Martin et al. (1997) found that disoriented rats could reliably find an escape platform in a water maze, but could not learn to reliably find the food location on a radial arm maze. However, in a follow-up study, Hynes et al. (1998) demonstrated that solution of the water maze problem was contingent on the animals being provided with a stable point of entry (POE) into the maze environment, even when they were not disoriented. The current experiment combines choice behaviour with electrophysiological recording to determine whether similar POE effects could be obtained in a dry land maze using an appetitive spatial learning task, and to determine the relative importance of map-parsing, environmental geometry, and path integration to the solution of the task.

1.2 Animals map space

The study of cognitive spatial mapping arose from the observation that the wide variety and flexibility of behaviour that animals exhibit in everyday life cannot be explained in terms of classical learning theory, according to which, responses are driven by specific stimulus inputs. For example, animals are capable of finding hidden caches of food in the absence of visibility of the cache or any markers immediately adjacent to it

(Gallistel, 1990). Similarly, rats are able to find a submerged escape platform in a water maze based solely on the position of the platform relative to distal features of the experimental room (eg., Morris, 1981). Tolman, Ritchie and Kalish (1946) observed that, faced with the blockade of the usual route to a goal, rats are capable of using entirely novel routes to reach the same destination. As suggested by Tolman et al.(1946), understanding how animals accomplish these tasks requires the assumption that animals store a representation or map of their environment in memory, in order to plan appropriate behaviour.

This is not to say that animals cannot or do not use non-spatial solutions to certain goal-oriented problems, because they can, and often do, where these solutions suffice (eg., McDonald & White, 1993, Martin et al., 1997). Problems which simply require the animal to approach a visible cue or make a stereotyped behavioural response such as making a left turn, do not require any form of spatial representation. For the solution of more complex spatial problems, however, it is clear that an animal must use an allocentric representation of the environment and the significant features in it, irrespective of the animal's position at any time. To successfully solve a complex spatial learning problem, an animal must 1) identify the environment it is in and use the appropriate map, 2) confer an orientation onto the map, and 3) monitor its position and direction relative to the map as it moves about the environment. It has traditionally been assumed that environmental recognition and map orientation are accomplished using salient visual features of the environment. Position tracking is often referred to as path integration, which involves use

of ideothetic (self movement) cues to calculate the vector (distance and direction) of an animal's displacement from an arbitrary starting point (see Gallistel, 1990, for a review).

1.3 Definition of terms

For the purposes of this paper, we will define the commonly used terms "map", "environment", "orientation", "disorientation", and "place" as follows. "Map" will refer to an animal's internal representation of space. "Environment" will refer to a given space the animal may create a map of. As suggested by previous work (eg., O'Keefe & Nadel, 1978, Gallistel, 1990), an animal's map of the world is composed of multiple maps of individual environments, as it seems unlikely that an animal would have a single, global map of everywhere it has ever been. "Orientation" will refer to the angular alignment of the map in use at any given time with respect to the animal's forward-facing head direction. In many ways, this is synonymous with what many researchers refer to as the animal's internal sense of direction. "Disorientation" will refer to any procedure which disrupts or produces "misorientation" of an animal's internal sense of direction with respect to the external world. Animals probably always have some notion of direction, but that sense may have varying degrees of accuracy. "Place" will refer to a particular location within an environment in the horizontal plane, as defined by its approximate Euclidean coordinates with respect to an arbitrary origin.

1.4 Approaches to the study of spatial mapping

1.4.1 Behavioural approaches The precise nature of spatial maps and the ways animals use them has been debated for some time. Over the past 20 years, there have been two main approaches to the study of spatial mapping. The traditional approach uses behavioural experiments, in which animals are trained on a spatial learning or memory task, and probe tests are used to determine the effect of certain manipulations on performance. Training typically takes place in either a water maze, in which animals have to find a submerged platform to escape (eg., Morris, 1981, Sutherland & Dyck, 1984), or a radial arm maze, in which animals have to visit the ends of the arms for food reward . Radial arm maze tasks can usually be divided into two categories. First, there are experiments in which all arms are baited and the animal must learn to visit each arm before reentering one from which the reward has already been retrieved (eg., Olton & Samuelson, 1976). This protocol is typically used for investigating working memory, as the animal must keep track of places which have already been visited. Then there are those experiments in which the animal must learn which of the arms contains the reward, in order to selectively visit it (eg., O'Keefe & Conway, 1980). Under these circumstances, as in water maze problems, experimenters are testing to see if the animal can learn the relationship between a reward and a particular place, as defined in this case by the baited arm's position relative to the experimental room, and/or a set of controlled visual cues.

1.4.2 Electrophysiological approaches. An entirely new method of investigating spatial mapping was introduced by O'Keefe and Dostrovsky (1971), when they

demonstrated that certain cells ("place cells") in the hippocampus of awake, freely moving rats seem to fire preferentially when the animal was in a particular place. This area of elevated firing was referred as the cell's "place field". This finding led O'Keefe to postulate that the hippocampus may be the brain structure responsible for producing and storing spatial maps, and initiated a long tradition of using recordings from place cells as an indicator of the status of an animal's spatial map. Additional evidence for the involvement of the hippocampus in mapping environments comes from studies in which lesions of the hippocampus and associated structures produce spatial learning deficits (eg., Long & Kesner, 1996, O'Keefe & Conway, 1980).

The majority of place cells studied to date have been pyramidal cells in the CA1 and CA3 regions of the hippocampus, although similar cells have also been identified in associated structures like the entorhinal cortex (Quirk et al., 1992), and the subiculum (Sharp & Green, 1994). Evidence suggests that any one place cell is part of a unique population of cells which collectively encode overlapping places in a given environment, and that a cell may participate in representation of any number of different environments, and be silent in others (O'Keefe & Speakman, 1987, Thompson & Best, 1989). It has been estimated that in any given environment, only 15% percent of pyramidal CA1 and CA3 cells will have spatial firing correlates (Thompson & Best, 1989).

The discovery of head-direction (HD) cells in the postsubiculum (Ranck, 1984), lateral dorsal thalamic nucleus (Mizumori & Williams, 1993) and the anterior dorsal thalamic nucleus (Taube & Muller, 1995) has provided a physiological substrate for an

animal's internal sense of direction; a critical component of path integration that can be investigated using techniques similar to those used for place cell recording. HD cells fire maximally when an animal's head points in a particular horizontal direction relative to gravitational forces, and are sensitive to disruption of vestibular input (Stackman & Taube, 1997).

Over time, it has become common practice for experimenters using place cell recordings to train animals to randomly sample regions of a circular or rectangular environment in search of food reward, in order to build accurate firing rate maps depicting the place in the environment where a given cell fires maximally; that is, it's place field. The position of place fields are taken as indicators of which map the animal is using, and the orientation of that map. If fields change the pattern of their firing dramatically, or if previously active cells stop firing in an environment, it is usually taken as evidence that the animal has called up a different representation or map. Similarly, HD cell recordings can provide information regarding the orientation of an animal's map, and sudden shifts of a HD cell's preferred firing direction often accompany entrance to a novel environment (Taube & Burton, 1995). With regard to the behaviour of place cells, it should be noted that Shapiro, Tanila and Eichenbaum (1997) propose a somewhat different model of hippocampal function, in which many place cells are responsive to the position of a particular cue in a cue array, such that rearrangement of the array can lead to a shift in the response of some cells that is not necessarily indicative of treatment of the environment as novel. Their results also suggest that the ensembles of hippocampal

neurons encoding a given environment do not exclusively encode the topological relationships between cues in an array. However, this is a contentious issue, and will not be further elaborated upon here.

1.4.3. Combining spatial learning tasks with cell recording. O'Keefe & Speakman (1987) performed an experiment in which choice behaviour was correlated with recordings from place cells while rats tried to locate the food-baited goal arm on a four-arm radial maze. The authors demonstrated that when an array of cues inside the maze was rotated across trials, rats who learned to use the cues to locate the food had place fields whose positions were defined by the orientation of the cue array. Dudchenko & Taube (1997) recently demonstrated that animals failed to find a food reward associated with the position of a visual cue on trials in which the preferred firing direction of HD cells did not rotate in accordance with the rotated position of the cue. It should be noted, however, few other experiments have compared choice behaviour on a spatial learning task and place field or HD cell data at the same time. As a result, there is still a paucity of evidence supporting the notion that place cell activity is predictive or indicative of behaviour in tasks where an animal must make an appropriate spatial response to reach a goal.

Given that animals can solve complex spatial problems, and that the existence of place cells provides a neural substrate for representations of an animals current position in it's environment, we will assume that animals are able to track their movements through the environment using some combination of self-movement cues. What follows,

then is a discussion of how animals select appropriate maps and confer orientation on them.

1.5 Recognition of environments: calling up the right map

Numerous researchers have suggested that spatial maps encode space in terms of the geometric relationships between objects in the environment, creating a Euclidean style map upon which the position of the animal and things like goals can be placed (Tolman, 1948, O'Keefe & Dostrovsky, 1971, Gallistel, 1990). Evidence that arrays of cues affect the recognition of environments (and hence the map the animal calls up) can be found in both the behavioural and electrophysiological literature. For example, in their original description of place cells, O'Keefe & Dostrovsky (1971) found that minor changes to the environment failed to alter the fields of some cells, but radical changes to the environment, like removal of a curtain that normally surrounded three sides of the screening table, caused a disruption of firing in some cells, which was accompanied by increased exploration of the environment, as if it was completely novel.

Kubie and Ranck, (1983) demonstrated that a cell with a field in one environment may not have a field in another, even when both environments have distal visual stimuli in common. Moreover, they found that when a cell had a field in more than one environment, the fields were often different in terms of size, cohesiveness, or relationship to the common distal visual stimuli. This suggests that the emergence of fields in a familiar environment that are qualitatively different from fields from previous recordings

in the same environment may serve as an indication that the animal is treating the environment as novel.

Suzuki, Augerinos, & Black (1980) trained rats to reliably forage on all arms of an eight-arm radial maze for food reward before re-entering a previously visited arm. Each arm was associated with a distinct visual cue suspended near it. On test trials, foraging was interrupted after three choices. The rat was then confined to the middle of the maze, and the remaining baited arms (and associated cues) were either rotated together, or rearranged. Rotation of the cues did not affect performance - the animals proceeded to visit previously unsampled arms, as identified by the cues near them. However, rearrangement of the cues caused the rats to treat the maze as if it was a new environment, and they failed to discriminate between baited and non-baited arms. This demonstrates that the animals were not simply remembering which cues were associated with previously visited arms. Rather, it appears as though they had formed a spatial map of the maze defined by the topographical relationship between the cues. When the cues were rotated, the rats continued to choose the previously unvisited arms, as defined by the cues adjacent to them, suggesting that the rats had simply rotated their maps in accordance with the new cue positions. When the cues were rearranged, however, the rats failed to preferentially choose previously unvisited arms, which may be taken as evidence that the rats were treating the maze as a new environment, and possibly calling up new maps.

It should be noted that access to visual cues is not required for the establishment of hippocampal representations of space. For example, Quirk, Muller & Kubie (1990) demonstrated that following exposure to an environment, if the lights were turned out, rats exploring the environment had place fields that continued to fire in their normal positions. More recently, Save (1998) found that blind rats develop normal place fields resembling those of sighted rats, when given repeated exposures to an environment in which they are permitted to explore.

It seems rats are capable of using means other than visual cues to identify the environment they are in, perhaps based on the sequence of events preceding placement in that environment, or perhaps determination of the overall shape of the environment through exploration. Unfortunately, however, no firm protocol has been established for determining if and when an animal calls up the wrong map.

1.6 Conferring orientation on maps

Behavioural and electrophysiological evidence supports the notion that animals can use visual cues to confer orientation onto spatial maps. For example, Suzuki, Augerinos, & Black (1980) demonstrated that rotation of the cue array resulted in a corresponding rotation in the pattern of responses the animals made under normal conditions, and allowed the animals to successfully sample all baited arms with a low error rate. O'Keefe and Conway (1978) trained rats to locate a food reward on a T-maze surrounded by an array of cues. Rotation of the cue array usually resulted in a

concomitant rotation in the position of a rat's place field. Additional evidence for cue control over the alignment of place fields come from experiments by Muller & Kubie (1987) and O'Keefe & Speakman (1987).

In recent years, however, more attention has been paid to the combined role of cues and the state of the animal's internal sense of direction in conferring orientation on spatial maps. While neither source of information exerts maximal control under all circumstances, evidence suggests that both can be used by animals seeking to orient their maps, and that in some circumstances, one source of input will override the other (see McNaughton et al, 1996, for a review). It should also be noted that inertial navigation systems are, by nature, prone to drift which produces errors proportional to the distance travelled (Barlow, 1964). As a result, it is reasonable to expect that reference to the physical environment is necessary to make occasional corrections for this drift, and to enable accurate navigation over longer distances.

The importance of an animal's internal sense of direction to the orientation of place fields is demonstrated by the finding that, when HD cells shift their preferred firing directions, hippocampal place fields tend to shift accordingly (Knierim, Kudrimoti & McNaughton, 1995). Furthermore, inactivation of HD cells in the lateral dorsal thalamus dramatically alters the firing rates and field positions of hippocampal place cells, and simultaneously disrupts optimal performance on spatial memory tasks (Mizumori, Miya, & Ward, 1994). This evidence indicates the importance of input from an animal's internal sense of direction to hippocampal representations of space.

Alyan & Jander (1994) trained mice to retrieve their pups from the center of an arena and then return to their nest on the periphery. The experimental room was rich in visual cues that could serve as landmarks. After over 100 training trials, the authors demonstrated that rotating the position of the nest relative to the landmarks by 90^0 did not prevent the mice from running to the center of the maze to get their pups, but when the time came to return to their nests, all the mice tried to return to the original nest location as defined by the landmarks. The mice did this, even though it meant that they did not return from where they had just come. This strongly suggests that, upon leaving their nest, mice with extensive experience in the arena were taking an orientation fix based on the landmarks. However, in the initial stages of learning, the mice ignored the shift in the apparent position of the landmarks caused by rotation of the nest, and returned to the nest by the same path they used on the outward trip. Moreover, when the room was darkened to prevent access to landmarks outside the arena, even experienced mice returned to the rotated nest location. In other words, it seems that mice initially take orientation from internally generated cues derived from self movement, but that experience leads to reliance on visual landmarks, and that in the absence of visual landmarks, they can return to using path integration.

Gallistel (1990) and Cheng (1986) proposed a model in which cues which convey information about the large-scale geometry of the environment exert a particularly strong influence on orientation. According to Cheng, disoriented animals fail to use local cues (coloured panels) in a rectangular box to locate a food reward. Instead, they rely on the

shape of the box, dividing their search time nearly equally between the correct location and the location 180^0 opposite, even when using local cues would easily permit disambiguation of these two geometrically equivalent choices. Similar results have been obtained with children (Hermer & Spelke, 1996).

Margules and Gallistel (1988) elaborated on this finding in an experiment in which they trained rats to dig for food in a particular place in a rectangular box. They demonstrated that, without disorientation, the rats did not make the rotational errors observed by Cheng (1988). Instead, the authors proposed that the rats used a stable orientation obtained from the geometry of the room to disambiguate the geometrically equivalent corners of the rectangular box, in order to find the buried food. However, when access to room cues was denied and animals were forced to use local cues inside the box, the animals divided digs between the correct position and the one 180^0 opposite. This demonstrates that animals can carry orientation derived from a larger environment into a smaller one in order to solve a spatial problem.

Rotating a box in which rats have been trained to forage for food results in an alteration of the properties of place fields - often causing them to stretch or split in two along the dimension in which the length of the box (relative to the experimental room) seemed to be enlarged (O'Keefe & Burgess, 1996). This finding suggests that in the absence of disorientation, place fields encode the relative distance of the animal from one or more surfaces in the environment, map orientation is not defined by the geometry of the box. It seems as though place fields in this experiment did not rotate in accordance

with the rotated box geometry, but remained oriented with the animal's internal sense of direction, with alterations induced by changes in the relative distances between surfaces along axes defined by the rat's direction sense.

McNaughton et al. (1996) propose a model in which path integration plays a primary role in spatial mapping and hippocampal function, while familiar visual cues are used to correct for drift in the path integration system. According to this theory, based on some of the evidence cited above, when an animal investigates a new environment, it initially builds a representation of the shape of the environment based on self movement, and aligned with the current status of the animal's direction sense at the moment the animal enters. According to the authors, it is only after fairly extensive exposure to the environment that objects like visual cues are added to the map. Numerous lines of evidence are cited in support of this hypothesis. For example, it has been shown that visual cues can come to control place fields which were established prior to any exposure to that cue (Quirk, Muller, & Kubie, 1990). Knierim, Kudrimoti and McNaughton (1995) trained rats to search for food in a circular arena enclosed by a curtain and containing a distinct visual cue. Animals were trained under two conditions: 1) with disorientation prior to release into the arena, or 2) without disorientation. They found that disorientation prevented the animals from learning that the cue had a fixed position from trial to trial, and as a result, the position of the cue failed to exert control of place fields. Moreover, animals trained under disorienting conditions had fields which rotated by unpredictable amounts from trial to trial, suggesting that the animal was using its internal sense of

direction to confer orientation to its map, even though its sense of direction was unstable across trials. It has also been shown that if an environment containing visual cues is rotated while the animal is in it, place fields only rotate with the cues after the animal has had time to become familiar with the environment. (McNaughton, Knierim & Wilson, 1994). On initial trials, however, the authors found that rotation of cues while the animal was exploring had no effect on the position of place fields. The message seems to be that early in exploration, spatial maps are based on path integration, and that cue control comes only after cue stability relative to a path integration-based reference frame has been established through repeated exposures.

1.7 Map parsing and orientation

Given that animals are able to use visual stimuli to identify environments, and able to use a combination of visual stimuli and internal direction sense to confer orientation onto their maps, we consider the following question: as an animal moves about its world, when does it call up new maps, and how are maps of adjacent environments linked in terms of orientation as the animal passes from one environment to the other?

Sometimes animals use multiple maps to encode what appears to be a single, unified environment. McNaughton (1996) has suggested that within a given environment, an animal may invoke several different reference frames (or maps), depending on the changing attentional requirements of the task, or even the trajectory the animal takes

through the environment. It has been demonstrated, for example, that in tasks requiring rats to run in two directions along a linear track, it is common to see place cells which have fields on the track when the animal moves in one direction, but the field either becomes silent or encodes a different place when the animal moves in the opposite direction (McNaughton, Barnes & O'Keefe, 1983). This strongly suggests that the rat is treating the track as a different place during each phase of the journey, as if travelling out and back in the track was one long trip on a u-shaped road, as opposed to a return trip over the same stretch. Such directionally-dependent place fields are typically only observed in environments where trajectories are constrained by the shape of the environment, and are uncommon in open-field environments (McNaughton, Barnes & O'Keefe, 1983). In this case, it could be argued that either the local view or the internal direction sense associated with the direction of travel determines which map the animal uses.

Given that animals use different maps for different environments, how is a consistent orientation maintained across maps? Poucet (1993) attempted to address this question by suggesting that each individual place representation may have it's own unique orientation which permits calculation of vectors to neighbouring environments, and that repeated exposure to adjacent environments eventually permits construction of a reference direction common to all environments. Without such a reference direction, Poucet (1993) argues, an animal who's sense of direction was aligned differently in environments A, B and C would have no trouble getting from A to B, and from B to C,

but would have difficulty orienting properly to travel from A to C. However, it is also possible that during exploration, animals arbitrarily assign an orientation to novel environments based on the current status of their sense of direction (Taube and Burton, 1995), and that provided 1) the same orientation is established on subsequent visits, and 2) the animal always uses the same path to reach the new environment, a mismatch in orientation between environments need not pose any problems for the navigating animal. In other words, a global reference direction may not be necessary across environments when the POE into each one does not vary.

Taube & Burton (1995) designed an experiment in which it was apparent that direction sense, as indicated by the preferred firing direction of HD cells, was not consistent between adjacent environments, even after repeated exposures. Rats were first trained to search for food in a cylindrical environment containing a distinct visual cue. Then they were allowed to enter a previously obstructed passageway, acquiring access to a novel rectangular environment, while the experimenters recorded from HD cells. Two interesting findings emerged. First, when an animal entered the passageway, it was not uncommon for a given HD cell to suddenly undergo a small shift in preferred firing direction away from that observed in the familiar cylinder, and that on subsequent visits to the passageway, whatever shift was observed on the initial exploration was maintained. Secondly, when the cue in the cylinder was rotated, the preferred firing direction of any given HD cell tended to shift accordingly. However, this did not prevent cells from reassuming their stereotypical preferred firing direction upon entering the passageway, so

that by the time they reached the rectangle, the shift induced by rotating the cue in the cylinder was no longer apparent. This experiment provides powerful evidence that animals can realign their sense of direction at the point of passage from one environment to another, and that this realignment is based on the animal's initial experience.

Moreover, the experimenters observed that over repeated cue rotation trials, when the animals returned to the cylinder, their HD cells became less and less likely to shift preferred firing direction to correspond with the rotated cue position. This may be interpreted as additional evidence that, given a mismatch between geometric (shape of passageway) and non-geometric (cue card) cues, animals will prefer to use geometric cues. Incidentally, it may be argued that it is the topography of the space, as defined by the way the animal is forced to move through the environment, which is being used to reorient the rat's internal sense of direction. Whether you say that an animal's sense of direction is aligned to the long axis of the rectangular passageway or that it is aligned to the direction in which the animal can move most freely is irrelevant. In cases where geometry defines the animal's behavioural space, either argument works.

1.8 Recent experiments suggest task-dependent map-parsing.

Recent experiments have demonstrated an interesting dichotomy in the results of disorientation on spatial learning, depending on the task the animals are required to perform. Dudchenko et al. (1997) trained rats to solve one of two types of spatial learning tasks inside a curtained enclosure: either an appetitive task on a traditional dry land

version of a radial arm maze, or an aversive task in a radial arm version of a water maze. In both conditions, a single large visual cue was hung on the curtain to facilitate learning. The experimenters found that disorientation prevented acquisition in most animals on the dry land maze, but not in the water maze. In addition, all animals which solved either task exhibited cue-control of choice behaviour, although non-disoriented animals still made a large number of correct choices when the cue was removed.

Martin et al. (1997) obtained similar results with disoriented rats trained on a four-arm radial maze. The animals failed to reach criterion on an appetitive spatial learning task, despite the presence of controlled visual cues, and whether or not a curtain was used to limit access to room geometry cues. In contrast, animals trained in an aversive water maze task were impaired by disorientation but eventually solved the problem. Removing access to room cues by enclosing the maze by a curtain also reduced performance, the disoriented animals being most severely affected. Despite this, four out of six disoriented animals still reached criterion, making these results consistent with Dudchenko's finding of a dissociation of the effects of disorientation on the radial arm maze versus the water maze.

More recently, Hynes et al. (1998) trained rats on a radial arm version of the aversive water maze task and found, again, that disorientation impairs but does not prevent spatial learning when access to geometric room cues was permitted - ie., with the curtain open. Closing the curtain, however, prevented solution of the water maze problem for disoriented animals, as well as most of the non-disoriented animals, unless they were

provided with a reliable single POE through the curtain. Each multiple POE animal was brought through the curtain at one of three randomly selected positions on each trial, and failed to solve the problem.

These results are consistent with the hypothesis that animals entering the curtained environment from the same direction across trials learn to use POE to confer a stable orientation on their spatial maps, permitting solution of the maze problem. We would expect this form of fix-taking to be particularly important at the moment of map-parsing, when the rat switches from one map (outside the curtain) to the next (inside the curtain). Multiple POE animals could not benefit from map parsing at the curtain, because if they had attempted to use POE to reset their sense of direction, both the goal location and the cues inside the curtain would appear to have been rotated by random multiples of 90^0 (the angle connecting adjacent POE's) between trials. Use of the cues for taking a directional fix would have resulted in a solution for multiple POE animals, but for most of the rats in this group, the obvious solution was not chosen. Only 25% of the multiple POE animals managed to solve the spatial learning problem. For these animals, cue-removal or rotation caused a disruption in performance, suggesting that their solution was based on the position of the cues. However, the disruption did not persist across testing days, suggesting that these animals, too, could rely on alternate solutions, perhaps by path integrating from the holding rack. Fixed POE animals were unaffected by cue rotation or removal, suggesting that their maps of the maze obtained orientation from their POE.

In support of this theory, Sharp, Kubie and Muller (1990) found that POE into an environment contributed to the gross angular position of place fields. Sharp et al. (1990) trained rats to search for sugar pellets in a cylindrical environment containing a single salient visual cue, which was surrounded by a curtain restricting access to other cues in the room. During training, the rats were always placed into the cylinder at a fixed point, and the cue was always positioned at three o'clock from the point of view of the overhead camera. The authors found that cue rotation alone did not produce a shift in place fields, nor did POE rotation alone. Combined rotation of both did cause fields to rotate. When a second cue identical to the first was added to the cylinder at 9:00 (180^0 opposite the first), POE alone determined the gross angular position of the rat's place fields, while 30^0 rotations of the pairs of cues produced corresponding shifts in the position of the fields. This result suggests that the cues in this condition served only to fine-tune the position of the fields.

1.9 The current experiment.

The current experiment was designed to determine whether Hynes et al.'s (1998) POE effect could be replicated in animals trained on a dry-land appetitive task, and whether what appeared to be POE-dependent map parsing was related to differences in ambient lighting inside and outside the curtain. Specifically, we wondered whether the POE effects observed by Hynes et al. (1998) were related to the fact that the room was brightly lit inside the curtain, and fairly dark outside. This may have been a factor in

determining whether the animals treated passage through the curtain as such a significant event that it warranted the resetting of map orientation.

In addition, the current experiment attempts to determine the degree of control internal direction sense and visual cues have over map orientation, in an effort to better explain the manner in which animals obtain solutions to spatial learning problems. Previous experiments in our laboratory (Martin et al., 1997, Hynes et al., 1998) were designed to see whether altering POE, access to room geometry, and disorientation could prevent learning or disrupt performance in animals which had learned. The current experiment was designed to specifically test the effect of disorientation and access to room geometry on the ability of animals trained under different POE conditions to use controlled visual cues.

Finally, this current experiment combines choice behaviour with place cell recording in the investigations described above. In this way, we hope to improve our ability to interpret our results, and to better comment on the validity of the assumption that the activity of hippocampal place cells is indicative of an animal's perception of its position and orientation in an environment. Is there any difference in the spatial maps of single (fixed) versus multiple (random) POE animals? Specifically, do animals deprived of a consistent POE use a unified representation of the maze, or do they have different representations depending on POE? Do place fields in learners and non-learners differ? Are non-learners really confused as to the environment they are in, or are they merely confused as to their orientation from trial to trial? Alternately, are non-learners actually

perfectly oriented and simply not choosing to maximise their rewards? These are just some of the questions we hope to be able to address by combining place cell recording with choice behaviour on a trial by trial basis.

In short, animals were trained to find food in a square box inside a curtained enclosure with a large visual cue. Some animals were given a fixed (single) POE, and others had a POE that was randomly chosen from one of four potential POEs on each trial. Probe tests were then carried out to determine whether animals which solved the problem were sensitive to changes to the POE used, the position of the controlled cue inside the curtain, disorientation outside versus inside the curtain, and varying combinations of these manipulations. Tests were also conducted to determine whether solutions to the maze were sensitive to the direction the animal faced upon release inside the maze, and inadvertent disorientation that may have occurred due to certain aspects of the procedure. In addition, place cell recordings were obtained from some animals following behavioural probe trials, to determine whether electrophysiological data could be used to draw the same conclusions as those obtained through the behavioural probes. Recording sessions were conducted in a similar manner to training and probe trials, with the addition of a 4-6 minute period after the animal made its first choice in which place cell recordings were collected. In this way, choice behaviour on a given trial could be compared with the status of the animal's place fields.

2. Method

2.1 Subjects

Sixteen male 36 to 39 day old Long-Evans rats (Rattus norvegicus), initially weighing between 102 and 116 grams (mean = 101 g), were used for this experiment. Between experimental sessions, the rats were individually housed in a colony room at 20±1°C, on a 12:12-hr light-dark cycle, with lights on at 8:00 a.m. Housing consisted of translucent plastic home cages (45.5 cm x 25.0 cm x 20.0 cm high) with wire covers and wood shavings covering the floor. Prior to surgery, each animal's cage also contained a 10.5 cm long piece of black PVC tubing with an inner diameter of 7.5 cm, which served as environmental enrichment. Throughout the experiment, water was available ad lib. Access to food (Prolab rat 3000, PMI Feeds, Inc., St. Louis, MO) was restricted in various ways throughout the experiment, as described below.

2.2 Apparatus

2.2.1 The experimental room. Experimental sessions were conducted in a 5.27 m x 4.52 m room with a large window on the west wall, a sink in the northeast corner, tables and recording equipment in the southeast corner, and an animal holding rack in the southwest corner. Light from outside the room was minimized by keeping the blinds closed at all times, and covering windows in the doors and near the ceiling with aluminum foil. A white, 226 cm diameter circular curtain hung from the ceiling to 71 cm above the floor in the north end of the room. The curtain had slits on the north, east, south

and west sides, with an additional flap of material covering the slit on the outside. Each slit served as a POE, allowing the experimenter access to the inside. A 93 cm high x 64 cm wide black sheet covering 32.5 degrees of arc served as a controlled visual cue, and was hung on the inside northwest surface of the curtain. Two speakers centrally mounted in the ceiling above the maze played music to mask audible cues inside the curtain. The experimental room is illustrated in Figure 1.

2.2.2 The maze consisted of a 79 cm x 79 cm square wooden box, positioned in the middle of the curtained enclosure, and mounted on casters to facilitate easy rotation. The floor of the maze was painted white and was raised 48 cm above the floor of the room. The walls of the maze were covered with white adhesive plastic and rose an additional 26 cm above the floor of the maze. Four 25 mm diameter and 13 mm deep circular depressions were made in the floor of the maze, one depression three cm from each corner. These depressions served as goal locations, in which 45 mg dextrose pellet rewards (P.J. Noyes Company Inc., Lancaster, NH) could be placed. Inverted steel cups, only a little larger than these depressions, served as lids, which had to be removed to gain access to the pellets. To prevent the rats from distinguishing between baited and non-baited goals by scent alone, each lid was packed with 20 dextrose pellets, held in place by a stainless steel mesh insert. Intra-maze cues were completely controlled by making all features of the maze symmetrical, and by placing the maze on wheels so it could be easily rotated between trials.

2.2.3 Illumination inside the curtain was always provided by a 104 cm diameter array of six 25-watt incandescent bulbs, suspended 198 cm above the floor of the maze. Illumination outside the curtain, when required, was provided by three linear arrays of four incandescent bulbs positioned in the southwest, southeast, and northwest corners of the room. These arrays had a mean output of 180 watts each. When the linear lighting arrays outside the curtain were turned on, mean illumination outside the curtain was 68 lux, while mean illumination inside was 72 lux. With the linear light arrays turned off, mean illumination outside and inside the curtain was 2.5 and 68 lux respectively.

2.2.4 Recording equipment. Recordings were obtained using a stereotrode made from a pair of insulated 25 m stainless steel fine wires (California Fine Wire Company, Grover Beach, CA) twisted together. A single strand of the same wire was used as a reference electrode. The ends of the wires to be inserted into the brain were cut to expose a small amount of bare wire. A small flame was used to burn the insulation off to about 5 mm from the tip of the other ends of the wires, to permit either soldering or silver-painting of the wires to a gold plated female amphenol pin (catalogue no. 19003-02, Fine Science Tools, Inc., North Vancouver, BC). The stereotrode and reference wires were mounted in a glass pipette with a tip pulled to a diameter of approximately 0.05 mm, such that the tip of the reference electrode and stereotrode extended beyond the end of the pipette by about 1.0 and 1.5 mm respectively . The impedance of the stereotrode and reference wires was typically between 0.5 and 2.0 MR. Ground wires were made from 100 m stainless steel ground wire with the insulation burnt off both ends and one end

soldered to an amphenol pin. For two animals, the free end of the ground wire was inserted in the pipette adjacent to the reference electrode. On all other animals, the free end was wrapped around a jeweler's screw that was implanted in the skull during surgery. Silver paint was added to improve the connection between the wire and the screw. Stereotrode, reference electrode, and (where applicable) ground wires were fixed in position in the end of the pipette using cyano-acrylate (Via-Chem Inc, Montreal, PQ).

The amphenol pins connected to the stereotrode, reference and ground wires were inserted into a strip that was then attached using epoxy to the side of a 3 cc syringe cut to a length of 18 mm. The pins in the strip served as a socket for attaching the FET plug during recording. The syringe formed a cylindrical microdrive shield large enough to be lowered over the microdrive during surgery, protecting it from subsequent mechanical shock. Attached to the shield was a smaller ring of plastic cut from the cap of a 26.5 gauge needle cover, which served as an anchor point for the field effect transistor (FET) plug, which relieved physical strain from the FET plug itself.

Microdrives were constructed from an 80 thread per inch stainless steel machine screw (model k-mx-080-8, Small Parts Inc. Miami Lakes, FL) with the slot and half the upper threads machined to a smooth surface, and a new notch made in the bottom of the screw. The smooth head of the screw was coated with a thin layer of petroleum jelly and fixed in dental cement along side a steel post of similar length, cut from an 18 gauge needle. Additional dental cement was poured around the bottom of the post and screw, where the threads were still intact, creating a microdrive with a solid base in which the

head of the screw could turn, and a top stage which could be lowered by inserting a jewellers screwdriver into the notch made in the foot of the screw to turn it. The top stage would slide down the smooth post, which served to prevent the top stage from twisting as the screw was turned. A brass cylinder (1.5 mm internal diameter, 2.0 mm external diameter) was attached to the base of the microdrive so that it was parallel to the screw and post, and extended approximately 1.5 mm below the base of the microdrive. This cylinder was approximately the size of the hole that would be made in a rat's skull during surgery to accommodate the electrode, so that when the cylinder touched the skull surface, the electrode was completely protected, and there was enough space between the skull and the base of the microdrive to pour in a stabilizing layer of dental cement. The pipette containing the stereotrode was then mounted to the top stage of a microdrive, so that the tip passed through the brass cylinder and extended an additional 2 mm beyond the end of it . One full turn of the screw lowered the stereotrode and reference electrode by 0.32 mm. One-eighth turns of the screw were easily managed, and lowered the electrode by approximately 0.04 mm. See Figure 2 for a diagram of the electrode and microdrive.

Stereotrode and reference signals from the animal's brain travelled via independent unity-gain FETs mounted in the FET plug and a 3.96 m length of hearing-aid wire (model VP3, Plastics One, Roanoke, VA) to a mercury swivel (Josef Biela Idea Development) centered 165 cm above the floor of the maze. From there the signal from each recording tip of the stereotrode was sent to a separate Grass RP5107E differential

amplifier, where the reference signal was subtracted out, and the differential signal was amplified 20,000 times and band-pass filtered between 600 hz and 3000 hz. The amplified signals were sent to a digital storage oscilloscope (model 400, Gould Electronics, Valley View, OH), an audio analyzer, and an A/D converter for analysis on a 386 pentium processor running Discovery V5.1 (DataWave, Longmont, CO).

The animal's position during recording sessions, as indicated by a red light emitting diode (LED) attached to the FET plug, was monitored using a video camera (series 3500, Computer) suspended 142 cm above the center of the floor of the maze and a video tracker (DataWave. Longmont, CO), and sampled 10 times per second using Discovery. One pixel represented an area of approximately 0.76 cm square.

2.3 Procedure

2.3.1 Group assignment. Rats were randomly assigned to one of four combinations of lighting condition and POE condition: light-light fixed point-of-entry (LF), light-light random point-of-entry (LR), dark-light fixed point-of-entry (DF), and dark-light random point-of-entry (DR). Each animal was assigned a goal in the maze which corresponded with one of the four depressions in the floor of the maze, and was always in approximately the same position relative to the world. For example, the goal for rat number 1 was always in the southeast corner of the box. Group, goal, and POE assignment were controlled so that each group consisted of four animals, and each animal within a group had a different goal and (in the case of groups LF and DF) a different

fixed POE. Light-light and dark-light groups were used to test the hypothesis that passage from a dimly lit environment to a brightly lit one might encourage map-parsing. Fixed-POE and random-POE groups were used to test the hypothesis that animals which map-parse at the curtain require a fixed POE through the curtain in order to solve the maze problem using path integration.

2.3.2 Pretraining. All rats underwent four days of pretraining to familiarize them with the task of moving metal lids to obtain dextrose pellet rewards. Pretraining was conducted in the colony room, and food was available ad lib. A piece of wood with three depressions, each containing a dextrose pellet, was placed in each animal's home cage for 5 minutes. On days 1 and 2, one of the depressions was covered with a metal lid. On day 3, two depressions were covered, and on day 4 all three depressions were covered. On day 1, each animal was given one 5-minute period to eat the pellets. On days 2, 3 and 4, the rats were given three 5-minute chances, separated by 10 minute intervals.

2.3.3 Training. The animal's spatial learning task was to learn the location of its goal (the baited depression) in the maze box. The cue panel always hung on the SW side of the curtain. Each animal underwent a single training session per day, along with the other animals from the same group. Each session consisted of 8 trials, with an inter-trial interval of approximately 5 minutes. During training sessions, animals in the group being trained were brought to the experimental room and transferred from their home cages to similar cages on the holding rack in the southwest corner of the room. For the first four

days of training, the rats were food deprived for 24 hours prior to each training session. On subsequent days, each rat was placed on a restricted diet of 4-8 pellets per day.

Prior to each training trial, the maze was rotated by a random multiple of 90^0, such that each side of the maze was always parallel with a side of the room. This prevented intramaze cues from ever having a consistent spatial relationship with the animal's goal, making such cues of no value for the solution of the spatial learning task. The animal's goal was then baited with a dextrose pellet, and all four depressions were covered with lids. On each trial, rats were removed from their home cages and carried through one of the four slits in the curtain, and the experimenter stood at one of the four sides of the maze. The route the experimenter and rat took from the rack to the maze was chosen so that they always travelled in a counter-clockwise direction around the curtain. Sample routes taken by the experimenter during training trials are shown in Figure 3.

Next, the rat was released in the center of the maze. For the first three days of training, rats were always released facing away from the experimenter. For the remaining training sessions, the rat was released facing one of four randomly selected sides of the maze, such that in any 8-trial session, they were released facing each side twice. The only exception to this rule was when animals exhibited response biases. A response bias was defined as making six consecutive non-spatial responses of a particular type in a row. For example, an animal that turned left six times in a row, regardless of the way he faced upon release, was said to have a response bias. When response biases were detected, the animal was never released facing a direction from which the favoured response would be

rewarded. This extinction procedure was continued until the animal made at least three responses of a different type.

Following release, all rats were given 60 seconds to make a choice. A choice was defined as moving one of the lids. A correct choice was defined as moving the lid over the animal's goal. The rat was removed from the maze and returned to the holding rack before a second choice could be made, whether the first choice was correct or not. On the return trip, the experimenter retraced the route taken from the rack to the maze on that trial. During training, it should be noted that the cue panel was always in the same position (the southwest side of the curtain) relative to any animal's goal, making it a stable indicator of the goal location. Eating the pellets was not required, but considered normal on trials when an animal made a correct choice. Each animal underwent training until it either reached criterion, or had undergone 432 training trials (54 sessions) - whichever came first. An animal was said to have reached criterion when it had made at least 18 correct choices in 20 consecutive trials. The exact training procedure for each group differed slightly, as described below.

For group LF, the room outside the curtain was illuminated by three arrays of lights, making the room illumination approximately the same inside and outside the curtain. Each animal was randomly assigned a POE, such that all four animals had a different POE. Each rat was carried from the holding rack and through the curtain at his respective POE. From there the rat was carried to a randomly selected side of the maze. The side of the maze the experimenter stood at was randomly selected, such that in any 8-

trial session the experimenter stood at each side twice. This ensured that the experimenter's position never had a stable relationship with the position of the animal's goal, so the experimenter could not be used as a visual cue. The rat was then released into the center of the maze, allowed to make a single choice, retrieved, and returned to his home cage, all as described above.

Group LR was treated in the exact same manner, except that each animal was randomly assigned a new POE on each trial, such that in a given session, each POE was used twice. Also, LR rats were not carried to randomly selected sides of the maze. Instead, the experimenter remained standing at the side of the maze adjacent to the POE and released the rat.

Groups DF and DR were treated exactly like groups LF and LR respectively, except that during training sessions the room outside the curtain was not illuminated. The result was that it was much darker outside the curtain than inside, and so all rats experienced a dramatic change in lighting on the way from the holding rack to the maze (and back) on each trial.

2.3.4 Probe trials. As each animal reached criterion, it underwent a battery of probe trials to determine the nature of the animal's solution to the maze. Only animals which reached criterion were probed. Animals which reached criterion will subsequently be referred to as "learners", and all other rats will be referred to as "non-learners". Animals from a given group that were being probed were brought to the experimental room and kept in their home cages on the holding rack in the southwest corner of the

room. The only exception to this was during disorientation probes, when rats were housed in metal cylinders as described below. Because animals reached criterion at different times, some animals underwent certain probes alone, and some with very few other animals in the room.

Each animal underwent a single probe session per day, each session consisting of 8 trials, with an inter-trial interval of approximately 1 to 10 minutes, depending on how many animals were being probed at that time. During the probe trial phase, food was made available ad lib for one hour daily immediately following experimental sessions, and each animal's weight was checked daily. This procedure ensured that the rats were hungry before probe trials, while allowing them to maintain their normal weight. During probe sessions, the rats' goals were baited with three 45 mg dextrose pellets.

In previous studies we have found that running many probe trials together often results in dramatic alteration of an animal's behaviour, sometimes causing animals which have acquired a solution to a maze problem to revert to making seemingly random choices. This makes it exceedingly difficult to collect sufficient probe data to do a meaningful statistical analysis. For this reason, in order to reduce the effect of probe trials on what the animal had learned up to this point, we used what we refer to as MINIMUM IMPACT PROBES, conducted as follows. (1) Single probe trials were embedded in a block of eight trials that made up the probe session, so that for every seven normal trials, the animal only experienced one trial in which something was altered. (2) Normal trials in the probe session were exactly like the training trials for that animal. (3) Probe trials were

embedded at random points in the session, but never occurred on the first trial of the day, when even very consistent performers were most likely to make an error. (4) When the manipulation conducted on the probe trial allowed us to make a prediction regarding the animal's choice, the depression corresponding with the predicted choice was baited along with the goal for the animal. As a result, should the animal be using the manipulated variable to locate his goal, acting accordingly would not result in failure to be rewarded, which might otherwise weaken the animal's reliance on that variable for solving the maze problem. If the animal made a correct choice, it was recorded as such. If the animal's choice was correct with respect to the manipulation performed (rotationally-correct choice), it was given a special code for analysis. Unpredicted incorrect choices were not rewarded. (5) Within a probe session, the remaining six normal trials (again, the first trial was not used) were randomly selected to serve as control trials. In this way, for each probe type, analyses could be conducted on three means: correct choices on control trials, correct choices on probe trials, and rotationally-correct choices on probe trials. (6) Probe trials were conducted in a procession from what we perceived to be the weakest manipulation to the most powerful. If weaker manipulations produced a dramatic effect in a given animal, more powerful probes were not conducted on that animal. Probes conducted were repeated in six subsequent probe sessions. (7) Probe trials were not conducted in a given session if the rat made more than two incorrect choices. If a probe had already been conducted and the number of errors later in that session exceeded two, the probe was not entered into the data set for that probe type, but counted as a spoilt

probe. This stipulation is based on the assumption that it is normal for an animal at criterion to make an occasional mistake, and perhaps an additional mistake on probe trials. However, should the number of errors exceed two, it becomes difficult to interpret what the normal behaviour of the animal is, and whether the probe conducted caused that behaviour to deviate from the norm.

We conducted 5 types of probes: POE-rotation (POER), cue-rotation (CR), POE+cue-rotation (POE+CR), disorientation (DIS), and disorientation+cue-rotation (DIS+CR). All learners underwent the first three types of probes, but not all animals underwent the other probes, because some animals died following surgery. During probe sessions, rats were kept on the holding rack in their original home cages. It should also be noted that on probe trials, regardless of the manipulation, a choice was only considered correct if the animal moved the lid over its goal, as originally defined. If the animal's choice corresponded with the manipulation, it was considered "rotationally-correct". Each type of probe is described in greater detail below.

POE-rotation (POER) probes were used for animals in groups LF and DF only. These probes were intended to determine whether the animals were map-parsing at the curtain, and resetting their sense of direction at the point-of-entry. On the probe trial, rats were brought through the curtain at one of the three alternate points-of-entry instead of their usual one. This was treated as a rotation in POE, so the rotationally-correct corner of the maze was baited in addition to the original goal for that animal. For example, if a rat's normal POE was side 1 and his goal was corner 2, then if, on the probe, the rat was

brought in through side 2, this was treated as a 90^0 POE rotation, so both corners 2 (the goal) and 3 (the rotationally-correct choice) were baited. Over six probe sessions, each animal experienced all three novel point-of-entry twice.

Cue-rotation (CR) probes were conducted on all learners. The purpose of these probes was to determine whether or not animals which had solved the problem were using the position of the cue to determine position and direction inside the curtain. Before the animal was brought through the curtain, the black panel was rotated clockwise by 90^0, 180^0, or 270^0. Over six probe trials, the curtain was placed in each position twice, and on each trial, the rotationally-correct corner was baited in addition to the animal's goal.

POE+cue-rotation (POE+CR) probes were conducted on all fixed POE learners which were unaffected by POE-rotation or cue-rotation alone. A rotation of POE was accompanied by a corresponding rotation of the cue. In this way, if either POE or cue but not both were required for making a correct choice, then rotating both should produce a predictable shift in the animal's choice. Again, on probe trials all three possible rotations were repeated twice, and both the animal's goal and the choice predicted by the combined rotation were baited.

Following POE+cue-rotation probes, some animals underwent surgery (see below), and not all survived. Therefore, some animals did not undergo the following probes.

Disorientation (DIS) probes were conducted next. These probes were intended to determine whether or not animals insensitive to the previous probes had acquired a maze

solution based on path integration from the holding rack. At the beginning of the probe session, the rat was transferred to a cylindrical metal cage 22.9 cm in diameter, 17.8 cm deep, and with a locking metal lid and wood shavings on the floor, and rotated by a random amount. On normal trials, the rat was removed from the cylinder and carried to and from the maze in the usual way for his group, then returned to the cylinder, the lid was replaced, and the cylinder was rotated by a random amount to disorient the rat. This change in the procedure for non-probe trials was considered essential, in order to show that housing and rotation in the metal cylinders was not sufficient to disrupt performance, provided the animal was removed from the cylinder while still outside the curtain. On probe trials, the rat was kept in the cylinder until the experimenter was inside the curtain, standing at the appropriate side of the maze and ready to release the animal - at which point, the rat was removed from the cylinder, placed on the maze in the usual manner, and allowed to make a choice. Then the rat was put back in the cylinder, the lid was replaced, and the experimenter retraced the path back to the holding rack, where the cylinder was rotated by a random amount. On both normal and probe trials, rotation after the trial, rather than just before it, reduced the likelihood that the rat might be still experiencing vertigo when removed from the cylinder.

Disorientation+cue-rotation (DIS+CR) probes were carried out next. These were conducted exactly like DIS probes in that the animal was not released from the metal container until it was inside the curtain, but in addition, the cue panel was rotated by a random multiple of 90^0, such that over six probe sessions each rat experienced the cue in

one of three novel positions twice. The purpose of these probes was to determine whether the rats performance would suffer when deprived of both a reliable internal sense of direction and stable position of the cue panel.

Extra CR probes were conducted last, to confirm that any animals who had not been sensitive to cue rotation in the past had not changed their strategy by the end of the probe phase. One additional feature of these CR probes was that, like the disorientation and DIS+CR probes, control trials from these last CR probes were conducted with rotation in the metal cylinder on the rack between trials.

For each subject exposed to each probe type, three means were calculated. The mean number of correct responses on control trials (M1) was calculated as an indicator of the normal level of performance for an animal over the time course of a given probe type, and was the value with which the other means were compared. The mean number of correct choices on the probe trial (M2) served as an indicator of the rat's ability to chose correctly on that probe type. The mean number of rotationally-correct responses (M3) served as an indicator of the degree of control the POE or cue-position had over the animal's responses. A response was rotationally-correct if it was not correct based on the normal position of the rat's goal in the room, but would have appeared correct to the animal had his sense of direction been determined by the manipulation performed. If for example the animal's POE was rotated 180 degrees on a probe, the rotationally-correct choice for that animal would be the corner diagonally opposite the rat's normally correct corner.

If an animal were insensitive to a particular type of probe, we would expect no significant difference between the number of correct choices on the control trials versus the probe trials (M1=M2), while rotationally correct choices would be minimal (M3<M1). Given sensitivity to the probe, we would expect a relative reduction in performance on probe trials (M2<M1). However, in order to argue that a probe actually controls choice behaviour, we would also want to demonstrate that there was no significant difference between the number of rotationally-correct choices on the probe and the number of correct choices on control trials (M3=M1), and also that the animal made more rotationally correct choices than correct choices on the probe trials (M3>M2). To this end, three one-way repeated-measure ANOVAS were conducted for each probe type to compare M1 vs. M3, M2 vs. M3, and M1 vs. M2.

2.3.5 Surgery. Both learners and non-learners underwent surgery to allow single cell recording from the CA1 region of the hippocampus via a microdrive-mounted stereotrode assembly. Rats were not food-deprived prior to surgery. All surgical procedures were conducted under sterile conditions. Rats were anesthetized with 1.5 ml / 100 g b.w. Avertin, administered i.p. in two doses: an initial 1 ml/100 g b.w., followed by a 0.5 ml / 100 g b.w. dose 5 minutes later. The second dose was accompanied by a 0.3 ml i.p. injection of atropine sulphate (0.6 mg/ml), to alleviate respiratory problems while the animal was under anesthetic. Ten minutes after the initial dose, the rat's head was shaved and placed in a stereotaxic device. An incision was made, and the skin, muscle and

membranes were retracted, exposing the skull from 3 mm anterior to bregma to 3 mm posterior to lambda.

A hand drill was used to make four small holes to accommodate the jeweler's screws which would anchor the dental cement and electrode assembly. A hole was drilled at coordinates 3.5-3.8 mm posterior and 2.5 mm lateral to bregma, the dura mater was removed, and the stereotrode was lowered until the electrode shield touched the surface of the skull. At this point, the tip of the electrode was at a depth below brain surface of between 0.50 and 2.0 mm. The microdrive was then fixed in place using dental cement, the microdrive shield was lowered, and more dental cement was poured to hold the entire assembly in place.

Flowers of sulphur were sprinkled around the incision as a topical antibiotic, and the rat was given a 0.25 ml i.m. injection of penicillin in sodium (120,000 units/ml) in each hindquarter. The bedding in the rat's home cage was replaced with paper towels, and acetaminophen in flavoured solution was added as an analgesic to the rat's drinking water, at a rate of 1 mg/100 g b.w./ml. The rat was then removed from the stereotaxic device, replaced in his home cage, and allowed to recover overnight under a warm lamp in a heated room (approx. 25^{0}C). On the following day, the rat was returned to the colony room and given at least four days to recover fully. During this time, the rat had ad lib access to regular food pellets and mash.

2.3.6 Recording. Following recovery, screening for cells began. Rats were screened one at a time, with no other rats in the experimental room. During screening,

animals were placed in their home cages on a small table which sat outside the north side of the curtain. On the first day of screening, the electrode was advanced to a depth of approximately 2.0 mm below brain surface, and the animal was left for at least 24 hours. On subsequent screening days, rats were typically given eight trials under training conditions to maintain choice behaviour. During these trials, the animal was connected to the recording equipment, and we looked for individual burst-firing units with waveforms at least three times as large as the noise level of the recording. If such units were observed, individual cells were isolated based on waveform characteristics compared across the two recording tips of the stereotrode, using the Discovery V5.1 software package (DataWave, Longmont, CO). Units were then monitored while the rat was allowed to roam the maze. If on any given day no suitable cell with place or directionally specific firing could be isolated, the electrode was lowered approximately 0.04 mm, and the screening procedure was repeated the following day.

If a cell exhibited place or directionally specific firing, several recording sessions were conducted, each lasting approximately five minutes. Each recording trial was conducted exactly like the training trials for that animal, except that after the animal's initial choice had been made and recorded, it was encouraged to roam the maze while either searching for randomly tossed dextrose pellets (for animals whose recordings did not contain muscle artifacts) or while being coaxed by hand (for animals in which chewing produced muscle artifacts which interfered with unit recording), as described above. At the same time, we recorded single unit potentials from the rat's hippocampus.

In this way, both behavioural and electrophysiological data were collected from each recording trial.

The first recording on any given day was considered a baseline recording, and was used to determine the position of the cell's firing field under normal training conditions. Subsequent recording sessions on any given day included probe tests to determine which environmental or training variables influenced the cell's firing. The main tests conducted were cue rotation, disorientation combined with cue rotation, and disorientation combined with removal of the cue. On average, one baseline and five test recordings were collected per day, until the place cell could no longer be isolated. If a cell was isolated long enough, additional probes were conducted, usually consisting of opening of the curtain to allow the animal access to visual cues in the room, and 45^0 rotation of the maze.

A computer program (Appendix A) was written to create firing rate maps based on the positions in the maze at which individual cells fired. The maze was divided into 10 by 10 bins. Each bin represented an area on the surface of the maze approximately 10 cm square (about 18 pixels), with the peripheral bins representing space occupied when the animal reared and projected its head over the edge of the maze. For each recording session, we calculated the number of spikes recorded from a given cell in each bin (S), the amount of time the animal spent in each bin (T), and the firing rate for that cell in each bin (S/T). The resulting firing rate map from the baseline recording session was then compared with rate maps from subsequent recording sessions to determine the effect of

different probes. Four clockwise-rotated versions of the baseline firing map were created - 0^0 (the original map), 90^0, 180^0, and 270^0. Rate maps from subsequent recordings on that day were correlated with these four baseline rotations to see 1) if any significant positive correlations were present, and 2) which rotated version of the baseline map produced the highest correlation. If, for example, a given recording produced the highest significant correlation with the 90^0-rotated map, the field was said to have rotated clockwise by 90^0.

On probe trials, place fields were categorized as having either 1) stayed in the same place, 2) rotated in a manner predicted by the manipulation, or 3) made an unusual response. An unusual response could be either a 90^0 rotation that did not correspond with the manipulation (suggesting disorientation), or a completely novel pattern of firing, suggestive of treatment of the maze as a novel environment. Results from recordings from all animals were combined to compare the proportion of each type of response on each type of recording session. We assume that unless the animal treats the box as a completely novel environment, its representation of space would not rotate by anything other than a multiple of 90^0, because of the powerful orienting influence of the box geometry. It seemed highly unlikely, for example, that an animal whose sense of direction was confused would think it was in a corner of the box, when in fact it was in the center. Similarly, we would not expect place fields which normally fire in a corner to suddenly start firing in the middle of a wall, unless the animal had called up a new map.

3. Results

3.1 Training

3.1.1 Acquisition. All rats quickly learned to knock the lids off one of the depressions within 60 seconds of release. Almost all animals, at some point during training, exhibited response biases. The most common bias was a preference to run forward to the nearest wall upon release, and turn either left (for some animals) or right (for others). Ten of the 16 animals were considered learners, having solved the maze problem and reaching criterion after between 247 and 419 trials (mean = 313.7). The remaining six rats had not reached criterion within 432 trials, and were considered non-learners. Of the 10 learners, five were fixed POE (two from group LF, three from group DF), and five were random POE (two from group LR, three from group DR). There was no effect of group membership on trials to criterion. A 2x2 ANOVA comparing mean trials to criterion revealed no difference between either the fixed POE vs. random POE conditions, and no significant interaction between POE condition and lighting condition $F(1,12)<.01$, $p>.05$. Similarly, a repeated measures ANOVA for the proportion of correct choices made by animals in each group over the first 27 days of training revealed no differences between groups based on POE condition, $M_{fixed}=.360$, $M_{random}=.292$, $F(1,14)=2.94$, $p>.05$, or lighting condition, $M_{light}=.311$, $M_{dark}=.341$, $F(1,14)=.49$, $p>.05$). After the first 27 days, some animals reached criterion, and the numbers of animals in each group became uneven.

3.1.2 Criterion from 3 vs. 4 release directions. During training, it was not uncommon to see rats which were nearly at criterion make persistent errors when released facing a particular side of the box, regardless of experimenter position - that is, sometimes animals appeared to have a solution to the maze facing three directions upon release, but not from a fourth. Figure 4 (top panel) illustrates trials to criterion for learners versus non-learners, and also shows the earliest trial on which a given animal would have reached criterion had we not considered trials on which they were released facing in the problematic direction. We refer to this as criterion from any three sides. Five of the 10 learners appear to have reached criterion facing three sides considerably sooner than they had reached criterion facing all four sides. Because animals which approached criterion from one or two sides only often did so as a result of response biases which were quickly extinguished, it was more difficult to study criterion from one or two starting orientations, and analyses considering these effects were omitted. Looking at the learning curves for individuals for trials on which they were released from each of the four possible starting orientations, it is easy to identify patterns of results arising from learning in general, failing to learn, response biases, and piecemeal acquisition of solutions. Some examples are presented in Figure 5.

A 2x2 ANOVA comparing trials to criterion from three sides versus four, and for learners versus non-learners indicates that learners, as expected, reached criterion significantly sooner than non-learners, M_{learn}=313.70, $M_{non\text{-}learn}$=432.00, $F(1,14)=35.75$, $p < 0.01$), criterion was reached in fewer trials on average from three sides than from four,

M_3=326.38, M_4=358.06, $F(1,14)=7.32$, $p<.05$), and that there was a significant group (learner vs. non-learner) by criterion (three sides vs. four) interaction, $F(1,14)=5.02$, $p<.05$). Analysis of simple effects showed that learners on average reached criterion from three sides significantly sooner than from four sides, $M_{learn3}=265.70$, $M_{learn4}=313.70$, $F(1,9)=10.82$. $p<.01$, whereas there was no difference for non-learners, $M_{non\text{-}learn3}=427.50$, $M_{non\text{-}learn4}=432.00$, $F(1,5)=1.00$, $p>.05$. These results are summarized in the bottom panel of Figure 4.

3.1.3 Disorientation from in-transit rotation. It also seemed as though, from trial to trial, animals performed worst when released facing the experimenter, regardless of where the experimenter stood. Considering that release orientation was related to the degree to which the animal was rotated by hand prior to release, we suspected that this rotation might have caused a subtle form of disorientation. Normally, the experimenter carried the rat facing forward. To release the animal facing away from the experimenter did not require any rotation. To be released facing left or right required turning the animal 90^0, and to face the animal towards the experimenter required a 180^0 rotation. A 2x3 mixed between/within ANOVA was used to compare the proportion of correct choices made by fixed vs random POE animals on trials where the animals were rotated by either 0^0, 90^0, or 180^0 prior to release. There was a significant effect for the degree of rotation, M_0=.411, M_{90}=.408, M_{180}=.332, $F(2,28)=5.46$, $p<.05$), but no effect for POE condition, M_{fixed}=.412, M_{random}=.359, $F(1,14)=1.32$, $p>.05$, and no interaction, $F(2,28)=.01$, $p>.05$). The effect or rotation is illustrated in Figure 6, from which it is clear that

turning the animal to the left or right did not produce a significant drop in performance, but turning it 180^0 did. A contrast ANOVA on the mean proportion of correct choices for 0^0 and 90^0 rotations combined versus 180^0 rotations revealed a significant effect, $F(1,14)=5.96$, $p<.05$.

At this point we decided that it might also be worthwhile to investigate potential effects of unintentional in-transit rotation on other legs of the path to the maze - namely, from the rack to the POE at the curtain, and, once inside the curtain, from the POE to the side of the box the experimenter stood at. The first analysis was a 1-way ANOVA comparing the proportion of correct choices made by fixed POE animals for each of four possible rotations encountered en route to the POE. Means for 90^0, 180^0, 270^0, and 360^0 rotations were .440, .546, .321, and .378, respectively. There was no effect of rotation, $F(3,4)=1.08$, $p>.05$. Mean proportion of choices correct for random POE animals undergoing the same rotations (means .371, .320, .380, and .365, respectively), analyzed using a within-subjects ANOVA, also failed to reveal any significant effect of rotation, $F(3,18)=.21$, $p>.05$, or lighting condition, $M_{light}=.326$, $M_{dark}=.392$, $F(1,6)=.61$, $p>.05$. There was no interaction, $F(3,18)=.01$, $p>.05$. A within-subjects ANOVA comparing the effect of rotations due to the path the experimenter took inside the curtain on the proportion of correct choices for fixed POE animals did not yield a significant effect of rotation, $M_0=.477$, $M_{90}=.379$, $M_{180}=.367$, $M_{270}=.428$, $F(3,18)=2.17$, $p>.05$, nor an effect of lighting condition, $M_{light}=.349$, $M_{dark}=.478$, $F(1,6)=1.95$, $p>.05$, nor a significant interaction, $F(3,18)=.16$, $p>.05$. Because trials with random POE animals always required

the experimenter to stand at the side of the maze adjacent to the POE, these animals never experienced rotation on that leg of the path. In sum, it appears as though only the final rotation prior to the release of the rat in its starting direction had an effect on performance.

3.2 Behavioural probe trials.

All learners performed well on POER, CR, POE+CR, DIS and extra CR probes. However, DIS+CR probes caused a sharp reduction in the proportion of correct choices animals made, and while some animals made frequent rotationally-correct choices, others tended to choose in a more random fashion. Results ranged from perfect cue tracking for subject 15, to apparently random choices for subject 18. As a group, however, the rats still made approximately 64% rotationally-correct choices on the DIS+CR probes - considerably more than the 25% that would be expected merely by chance. Results of probe trials are summarized in Figure 7, and a detailed analysis of the results follows below.

One-way repeated measure ANOVAS showed that there was no significant difference between the proportion of correct choices on probe trials versus control trials for POER probes, $M_{control}$=.910, M_{probe}=.800, $F(1,4)$=3.79, p>.05, CR probes, $M_{control}$=.858, M_{probe}=.783, $F(1,9)$=2.10, p>.05, POE+CR probes, $M_{control}$=.890, M_{probe}=.654, $F(1,3)$=2.74, p>.05, DIS probes $M_{control}$=.870, M_{probe}=.806, $F(1,5)$=2.11, p>.05, and extra CR probes, $M_{control}$=.833, M_{probe}=.898, $F(1,5)$=1.11, p>.05. However, there was a significant difference

between the proportion of correct choices on control trials versus DIS+CR probe trials, $\underline{M}_{control}$=.927, $\underline{M}_{probe}$=.139, $\underline{F}(1,5)$=109.50, $\underline{p}$<.01.

One-way repeated measure ANOVA's revealed a significantly smaller proportion of rotationally-correct choices on probes versus correct choices on control trials for POER probes, $\underline{M}_{control}$=.910, $\underline{M}_{probe}$=.067, $\underline{F}(1,4)$=425.30, $\underline{p}$<.01, CR probes, $\underline{M}_{control}$=.858, $\underline{M}_{probe}$=.067, $\underline{F}(1,9)$=394.10, $\underline{p}$<.01, POE+CR probes, $\underline{M}_{control}$=.890, $\underline{M}_{probe}$=.269, $\underline{F}(1,3)$=25.01, $\underline{p}$<.05, DIS+CR probes, $\underline{M}_{control}$=.927, $\underline{M}_{probe}$=.639, $\underline{F}(1,5)$=8.24, $\underline{p}$<.05, and extra CR probes, $\underline{M}_{control}$=.898, $\underline{M}_{probe}$=.111, $\underline{F}(1,5)$=208.82, $\underline{p}$<.01. On DIS probes, rotationally-correct choices were equivalent to correct choices, as there was no manipulation of cue position or POE. Although rotationally-correct choices were apparent on the DIS+CR probe trials, animals still made significantly fewer rotationally-correct choice on DIS+CR probes than correct choices on control trials.

Finally, we looked at differences in the proportion of correct choices animals made on probe trials, versus the proportion of rotationally-correct choices. One-way repeated measures ANOVA's showed that animals made significantly more correct choices than rotationally-correct choices on POER probes, $\underline{M}_{correct}$=.800, $\underline{M}_{r\text{-}correct}$=.067, $\underline{F}(1,4)$=74.46, $\underline{p}$<.01, CR probes, $\underline{M}_{correct}$=.783, $\underline{M}_{r\text{-}correct}$=.067, $\underline{F}(1,4)$=74.46, $\underline{p}$<.01, and extra CR probes, $\underline{M}_{correct}$=.898, $\underline{M}_{r\text{-}correct}$=.111, $\underline{F}(1,5)$=42.25, $\underline{p}$<.01. Rotationally-correct choices were more common on the DIS+CR probe trials, $\underline{M}_{correct}$=.139, $\underline{M}_{r\text{-}correct}$=.639, $\underline{F}(1,5)$=13.50, $\underline{p}$<.05, and there was no difference between the proportion of correct

choices and rotationally-correct choices on the POE+CR probes, $M_{correct}$=.654, $M_{r\text{-}correct}$=.269, $F(1,3)$=2.59, p>.05.

3.3 Cellular recording

3.3.1 Surgical outcomes and histology. Surgery was performed on 12 of the 16 animals trained. Of these, successful place cell recordings were obtained from three (subjects 9, 13, and 15). In subjects 1, 2, and 6, single units were observed that appeared to have the waveform and burst firing characteristics of hippocampal pyramidal cells, but no coherent place fields were identified for any of them. No good cellular recordings were obtained from subjects 3, 5, and 16, due to unidentified mechanical failure at some point in the electrode assembly. The remaining three animals (subjects 4, 7, and 8) died before place cell recordings could be obtained. Of the three animals from which successful recordings were obtained, two were learners (subjects 9 and 15) and one was a non-learner (subject 13). Recordings were taken from two distinct place cells in subject 9, one in subject 13, and three in subject 15. In addition, what appeared to be a HD cell was recorded from subject 9.

After all recordings were collected, all animals were killed, and glycogen phosphorylase and Nissl-staining was performed on alternating 25 m coronal sections of the brains of subjects 1, 2 and 6, 9, 13, 15 and 16. In subjects 9, 13, and 15, the electrode passed through the CA1 cell layer of the hippocampus, as illustrated in Figure 8. Representative waveforms are illustrated in Figure 9. Placement was also found to be

good for subjects 1, 2, and 6 (not pictured). Placement in subject 16 (not pictured) appeared to have been somewhat anterior and lateral, missing the hippocampal formation.

3.3.2 Main probes: stability and cue control of place fields. The effects of the three main types of probes conducted are illustrated in Figure 10. On all trials in which no manipulation was performed (6 cells, 3 rats, 27 trials), rats' fields remained unchanged relative to the baseline recording for that day. On CR probes (4 cells, 2 rats, 16 trials), place fields remained in the baseline position on most trials (68.8%), there was some evidence of cue-tracking (18.8%), and there were a small proportion of unpredicted rotations (12.5%). On the DIS+CR probes (2 cells, 2 rats, 8 trials), fields never appeared in their normal location, but rotated by the same amount and in the same direction as the cue, on 75.0% of trials. On only 25.0% of these probe trials did fields rotate in an unpredicted manner. In the absence of the cue card (1 cell, 1 rat, 8 trials), disorientation produced fields that were in the normal position on 50% of the trials, and rotated by some multiple of 90^0 on the other 50%. Figure 11 shows a stable field for one of subject 15's place cells which undergoes rotation on a DIS+CR probe trial. The results of a correlational analysis of these fields is shown in Table 1.

3.3.3 Additional probes: open curtain and 45^0 maze rotation. We recorded place fields for two cells from subject 15 and one cell from subject 9 during trials (1 trial per cell) on which the curtain was drawn back from around the maze so that the entire curtain occupied the space normally occupied by the cue card alone. The cue card was not visible on these trials. One cell's field was unchanged by this manipulation, one's was rotated 90^0

clockwise, and the third exhibited an unusual field that did not correspond with any rotated version of the baseline recording for that cell. Disorientation alone (with the curtain in place) resulted in a rotated field for subject 15 (1 trial), and no effect for subject 9 (2 trials). Simple removal of the cue produced no change in subject 15's place field (1 trial).

Recordings were obtained from one cell from each of subjects 15 and 9 on trials on which the maze was rotated by 45^0. It is worth noting that on these trials, place fields never maintained their firing position relative to the cue. Fields either rotated (relative to the camera view) with the box geometry, or stopped firing almost completely. For subject 15, this manipulation resulted in a dramatic reduction in the firing rate of the cell on one trial, and what appeared to be a 45^0 clockwise rotation of the field on another two trials. Clockwise rotation of the maze halfway through a recording session also seemed to produce a concomitant rotation in the animal's place field. Subject 9's place field rotated 45^0 counter-clockwise on the maze-rotation trial.

3.3.4 Correlational analysis of place fields. On all trials but the 45^0 maze rotation trials, correlational analysis of firing rate maps compared with rotated versions of the baseline firing rate map for that day supported our subjective impression of what any given place field was doing. That is, there was always only one significant positive correlation, and it always corresponded with the rotation that we had assigned the field on a qualitative basis.

It was difficult to test that a field rotated by 45^0, because there is no simple way to create a version of a rate map from a square environment rotated by that amount. Any such rotation results in a map that omits space covered by the original map, and includes space omitted by the original. As a result, correlations with all rotated versions of the baseline map tended to be very low. For this reason, subjective observations, which we feel to be accurate, had to be used to determine place field positions on 45^0 maze-rotation probes. Figure 12 illustrates firing rate maps from a place cell in subject 9, on three consecutive trials from one afternoon. Recordings from control, 45^0 maze rotation, and no-curtain trials are shown. The corresponding correlations are presented in Table 2. A complete set of correlational analyses for all days of recording is presented in Appendix B.

3.3.5 Mismatches between choice behaviour and place field location. Choices on probe recording trials were compared with the choice made on the baseline recording trials (or the normal correct choice, for learners) to determine the amount by which choices on probe trials were rotated. This was compared with the degree of place field rotation on the same probe trial. The percentage of trials on which choice rotation matched field rotation was calculated for each animal. There was a 44.4% match over 18 recordings from subject 9, a 10.0% match over 10 recordings from subject 13, and an 85.7% match over 42 recordings from subject 15. The degree of match appeared to have less to do with which probe was being performed than with which animal the recording was obtained from. It was apparent that even in the absence of manipulations,

choice/field match was good for subject 15 was good, while choice behaviour from animals 9 and 13 was erratic, even though their place fields were relatively stable. Despite variability in choice behaviour, a place cell from subject 13 (a non-learner), exhibited a stable place field. The field is illustrated in Figure 13, and the corresponding correlation matrix can be found in Table 3.

3.3.6 A head direction cell. On the first day of recording from subject 9, we recorded multiple unit action potentials from what appeared to be a bundle of axons. We were successful at isolating signals from a single axon or group of axons which seemed to fire exclusively when the rat was facing northwest while in his home cage on the screening table, regardless of his position in the cage. We conducted several training-condition-type trials with the animal, on which after making a choice, the animal was held by an assistant and passively rotated through 360^0 in 45^0 increments in order to determine the preferred firing direction (directional tuning) of the cell in the maze, and whether there was a correlation between choice and directional tuning. Unfortunately, both choice behaviour and directional tuning changed dramatically from trial to trial, and there was little, if any, correlation between choice and the direction in which the cell fired most frequently in the maze. The instability of the cell's preferred firing direction across trials is illustrated in Figure 14.

More consistent results were obtained on a series of unrecorded trials on which the rat was simply carried inside the maze and back to the screening table, slowly, several times. It became clear that as the animal was walked around the curtain from the

screening table to it's POE, the preferred firing direction of the cell changed from northwest to southeast, with intermediate orientations observed en-route. Upon passage through the curtain, the cell continued to fire when the rat was facing southeast, and kept that preferred firing direction when the rat was placed in the maze.

Even more intriguing was the result when the path was retraced, and the animal was brought out through the curtain, around the outside, and back to the screening table. When travelling in this direction, the cell maintained it's directional tuning to the southeast orientation, even when replaced in it's home cage on the screening table. Then, over the course of about 10 seconds, a shift was observed as the cell returned to firing when the animal faced northwest. In order for this shift to occur, it appeared necessary for the animal to have a few seconds in which to explore it's home cage again. If it was encouraged to stay facing southeast, the cell would not shift back to it's original northwest directional tuning. This procedure was repeated several times, with the same results (as illustrated in Figure 15) each time. By the following day, we could no longer reliably isolate any one cell/axon, and the firing rate appeared nearly continuous, regardless of the direction in which the animal faced. Histological results suggested that the HD cell was recorded at coordinates 3.5 mm posterior, and 2.2 mm lateral relative to bregma, and 2.34 mm below the brain surface.

4. General Discussion

4.1 Absence of a POE effect.

Learning in this experiment did not appear to be dependent upon or affected by provision of a fixed point of entry versus random point of entry into the curtained environment. Unlike the water maze results obtained by Hynes et al. (1998), our results suggest that this particular spatial learning task did not produce animals which reset their map orientation with respect to POE. Similarly, altering the lighting conditions did not affect the likelihood of map orientation resetting, as there was no effect of lighting condition on learning on either the fixed or random POE animals. In recordings obtained from two random POE rats, there was no evidence of POE-dependent place fields. These findings are consistent with Hynes et al.'s (1998) hypothesis that it is the aversive nature of submersion in water that produces map resetting at the last significant event (passage through the curtain) before submersion, and that POE-based solutions in dry-land appetitive tasks may not be favoured.

4.2 Rats oriented using internal direction sense and visual cues.

The most dramatic finding of this experiment was that neither POE rotation, cue rotation nor disorientation alone prevented rats from choosing correctly during probe trials, while disorientation combined with cue rotation produced a dramatic reduction in the animal's number of correct responses, along with fairly reliable cue tracking in some

animals. As a group, all animals made significantly more rotationally-correct choices than correct choices on DIS+CR probes, even though they also made fewer rotationally-correct choices on the probe trials than correct choices on the control trials. Place cell recordings provided evidence that place fields and choice behaviour were affected in a similar manner by CR and DIS+CR probes - that is, it took a combination of disorientation and cue rotation to get any amount of reliable cue tracking by place fields. Sharp, Kubie and Muller (1990) found similar evidence of the combined but not individual control of visual cues and internal direction sense on place fields. The finding that cellular activity and behaviour are similarly affected by experimental manipulations has been previously demonstrated for place cells (O'Keefe & Speakman, 1987) and HD cells (Dudchenko & Taube, 1997). Results from the repetition of the CR probes at the end of this phase of the experiment confirmed that the rats had not switched to exclusive use of the cue panel for orientation.

As previously hypothesised by McNaughton et al. (1996), these findings suggest that rats build spatial maps onto which orientation can be conferred from multiple sources. Our rats had the option of either using an orientation obtained from the experimental room and maintained by path integration as they were carried to the maze, or, once inside the maze, they could orient using the cue panel. However, in the event of a path integration/cue panel mismatch (CR probes), the path integration information took priority, unless explicit attempts were made to disorient the animals (DIS +CR probes). It appears that each animal's perceived state of its direction sense was used to decide which

source of information would be used for map orientation. Contrary to the findings of McNaughton, Knierim & Wilson (1994), path integration took priority in our experiment, despite the fact that the animals had months of experience in the training apparatus with exposure to the cue and without explicit disorientation.

It is also evident that it was, specifically, disorientation inside the curtain which was responsible for the shift to cue-use observed on DIS+CR probe trials. Disorientation outside the curtain on control trials produced no decrement in performance, and neither did cue rotation combined with disorientation outside the curtain on the extra CR probes. It is unlikely that the rats were simply solving the maze problem by path integrating from the rack, because while correct estimates of distance might be made, it is difficult to imagine an animal knowing which direction the goal was in, having just been released from an opaque container which had been fairly vigorously rotated a few moments earlier. Clearly, upon removal from the metal cylinder, the animal has to establish a sense of direction from something in the room, and, in keeping with Gallistel (1990), geometry seems to be a likely candidate. We conclude that, provided the animals had the opportunity to view the experimental room on the way to the curtain, prior disorientation did not prevent the rats from conferring a stable orientation based on room geometry onto their maps. This orientation was maintained by the rat as it was carried through the curtain, to a side of the box, and released, providing a reliable solution to the maze problem.

It should be noted that once orientation is obtained using room geometry, all the animals would have to do to remain oriented in the maze is maintain a reliable sense of direction while being carried from the holding rack to the maze. Path integration per se is not really necessary. Seeing as this is simpler than assuming that the rats have actually also tracked their absolute displacement from the holding rack, we will assume that this is how solutions were obtained.

4.3 Stability of place fields relative to choice behaviour.

The impressive stability of place fields on trials where no manipulation was performed suggests that on these trials, the three animals tested were reliably oriented regardless of starting orientation in the box, the side of the box the experimenter stood at, and, in the case of the two random POE subjects, the POE used. It also suggests that these rats use a single map of the box across trials, as opposed to multiple maps individually oriented with respect to trial-specific conditions like the direction faced upon release.

On disorientation+cue removal trials, place fields remained in their normal positions on 50% of the trials, as opposed to the 25% expected if the animals had been truly deprived of any source of orientation once inside the curtain. This suggest that the place fields we observed may be, to some extent, sensitive to uncontrolled background cues in the environment which may enable the rat to confer orientation onto its map. On the other 50% of trials, fields appeared to rotate by random multiples of 90^0, suggesting that box geometry had a powerful influence in orientation, even when the animals were

not intentionally disoriented. In no instance did we observe anything resembling remapping on these trials.

For subject 15, choice behaviour predicted place field location fairly reliably, and both choices and place field locations remained relatively unchanged across control trials. It is worth noting, however, that occasional dissociations were observed. Sometimes the animal's choice would change for no apparent reason, even though the field remained the same. Other times, cue rotation would affect the field, but the rat's initial choice would be correct. Nearly every combination of results was obtained at least once, but the choice/field match was still 85.7% for subject 15.

For subjects 9 and 13, on the other hand, there was a very poor match between choice and place field location, even though place field location itself was fairly predictable. So how do we explain stable or predictable fields in animals with such unpredictable choice behaviour? In subject 9, a learner, we suspect the explanation may be that as the rat became accustomed to searching for randomly tossed sugar pellets in the maze, making a correct choice in the first 60 seconds of the recording session may become less and less of a priority for the animal, until after several blocks of recording trials, heroic efforts were required to get the animal to regularly chose correctly, even on non-recording trials. It should be noted that the better choice consistency observed with subject 15 may have been due to the fact that this animal had experienced both behavioural probe sessions and cell recording sessions on the same day, increasing the relative exposure of this animal to normal behavioural choice trials on days when random

search behaviour was also required. Satiation may have also played a role in the change in subject 9's behaviour.

All of this suggests that, ideally, the same minimal impact probe procedures employed during behavioural probe sessions should have been applied to recording sessions as well, with single probe sessions embedded in a block of eight control trials in which there was no pellet chasing. Of course, this would have greatly reduced the number of recording sessions which could be conducted in one day, and would have contributed to satiation of the animal as the day went on. The benefit, presuming that the cell could be isolated long enough to collect a useful amount of data, would be that the impact of pellet-chasing trials would be minimized, promoting more reliable choice behaviour throughout the recording phase, and adding an additional dimension to the interpretation of place field data. Subject 13 on the other hand, is a non-learner, from whom erratic choice behaviour is expected, but stable place fields are not. Perhaps if we had reliably recorded from this animal's place cell for more than one day, we would have seen the field become erratic with time, or perhaps even become more stable on some days than others. However, in the absence of an extended recording period for this animal, we must express concern about the validity of choice behaviour as an indicator of spatial knowledge in animals which do not make predictable spatial choices. The possibility remains that this animal knew exactly where it was in the maze during training, but either did not learn that the food was always in the same place, or chose not to make choices based on that knowledge.

Qualitative analysis of the behavioural choices subject 13 made on training trials (see Figure 16) confirms that the animal had a response bias. Presence of a response bias does not permit interpretation of an animal's spatial knowledge of an environment, but it does raise the question of whether some of our non-learners would have been labelled learners using other criterion which was insensitive to first choice, like dwell time in the correct corner on unbaited trials.

4.4 Radical environmental changes produce reorientation or remapping

When we rotated the maze by 45^0, we were creating a dissociation between the geometry of the larger room and the geometry of the maze. As a result, we saw fields rotate with the box geometry, and, in one instance, change in a dramatic manner suggesting remapping (the cell stopped firing in the misaligned maze). While we assume that solution of the maze problem required carrying some stable map orientation into the maze from outside the curtain, it is also apparent from these results and previous studies that the geometry of the animal's immediate environment exerts a very strong influence on the orientation of an animal's map, and perhaps, the animal's ability to identify the environment it is in.

In agreement with our findings, when the shape of a rectangular box was changed so that the box appeared rotated with respect to the geometry of the larger room, O'Keefe & Burgess (1996) noted that fields normally found in the box often move, stretch or split to maintain a constant position relative to one or two of the walls of the box, and

sometimes stop firing as if the animal were in a completely novel environment. In the current experiment, in order to maintain a constant relationship with the inner surfaces of the maze, place fields would not have to stretch or split, but would simply have to rotate with the box relative to the larger room - which they did. This is also consistent with the findings of Margules & Gallistel (1988), who found that when the geometry of the box was misaligned with respect to room geometry, animals tended to switch to smaller scale geometry (the box) for making choices.

When the curtain was withdrawn, we observed three different results from three different cells - field normal (suggesting a map with normal orientation), field rotated (suggesting disorientation), and field different (suggesting remapping). It is difficult to draw any particular conclusions from these results, other than to say that such a drastic alteration to the immediate environment in which the animal experiences the maze seems to influence the manner in which it maps the maze itself. Obviously, in the novel-field case, as in the cease-fire case during 45^0 maze rotation, being in the maze was not in itself sufficient to produce normal activity in the place cell. This is in accordance with similar findings by O'Keefe & Dostrovsky (1971). The series of events leading up to placement in the maze, and/or the appearance of the environment outside the maze walls, may contribute to recognition of the maze environment as the familiar one in which the animal had been trained.

4.5 Unanticipated sources of disorientation.

If we look more closely at the combined POE+CR probes, another interesting finding emerges. On most probes (except DIS+CR probes) rats make significantly more correct choices than rotationally-correct choices. However, during POE+CR probes, there is no significant difference between the proportion of correct choices and the proportion of rotationally-correct choices. On these probes, three of the five animals tested showed what appeared to be a noteworthy reduction in performance, and at least one showed some evidence of cue tracking. Just as rotation inside the curtain may be a form of subtle disorientation, the results suggest that while overall performance does not differ significantly from performance on control trials, the animals do make more errors than normal, which may or may not result in increased use of the cue panel for orientation. While statistically this is a non-effect, it is tempting to think that bringing the animal in from an atypical POE is a subtle enough form of disorientation to cause the animals to make errors, but not explicit enough for the animal to make a decision to use the cue rather than internal sense of direction for orientation inside the curtain.

It was also interesting to note that the final rotation accompanying the process of positioning the rats to face one of the four sides of the box also had an effect on the rat's ability to solve the maze, as measured by the proportion of correct choices the animal made on trials in which it was turned by varying degrees. If incidental disorientation while placing the animal in the maze influences the rats' ability to solve the problem, and intentional disorientation inside the curtain promotes reliance on the cue, it was surprising

that we found no effect on correct choices for the amount of rotation the animal experienced as the experimenter walked around the maze to the side from which the rat was to be released. However, the particular potency of the final rotation just prior to release may lie in the speed at which the animal was rotated. For the experimenter to walk to the opposite side of the maze (180^0 rotation) took about three to four seconds, while turning the animal 180^0 prior to release took only 0.5 seconds or less. It may be that rotations at this speed, but not the more gradual kind produced by walking around the curtain and the maze, are capable of producing subtle disorientation. Unfortunately, whether or not this form of disorientation would affect cue control of choice behaviour or place fields was not explicitly tested, although a qualitative evaluation suggested that it did not.

4.6 Piecemeal map construction.

If solution to the maze problem was a "eureka"-type event, we would have expected performance from all starting orientations to improve rapidly at the same time. This was, in fact, observed for some animals. However, the finding that many learners obtained solutions when released facing three sides of the maze before reaching criterion from all four suggests that these animals are building maps of the maze environment in a piecemeal fashion, from one starting orientation at a time. Had explicit measures not been taken to extinguish behaviours that resembled response biases, it may have also been relatively easy to quantify emergence of solutions from one and two starting orientations

as well. We believe, however, that at least in some cases, animals which were developing piecemeal representations of the maze were thwarted by extinction procedures. Ironically, this also may have contributed to the difficulty some animals had in reaching criterion.

In any case, it was impossible to confuse solutions from three release directions with response biases, because such a solution requires that the animal consistently make at least three different types of response, and to make them at the appropriate time. For this reason, the fact that many of our learners reached criterion from three sides significantly sooner than they did from all four provides strong evidence of piecemeal map construction. Poucet (1993) has suggested that animals build coherent maps of environment by putting together representations based on particular local views, even though, ultimately, orientation is not based on any particular local view. Ellen, Soteres & Wages (1984) previously demonstrated that animals were able to build complete representations of a complex environment in a piecemeal fashion. The authors showed that when rats were exposed to Maier's three-table-maze problem apparatus two parts at a time, 60% of the animals successfully navigated all three tables on test trials. All animals allowed to explore all three tables every day solved the problem, while none of those permitted to explore only one table per day reached criterion, although it was suggested that, given more time, even the 1-table group may have learned to integrate their table-specific representations of space. Whether intermediate maps are tied to a particular orientation of the rat's internal sense of direction upon release, a particular local view, or a combination of both is uncertain.

In the current experiment, it was impossible to tell from the behavioural data whether our learners had a single unified map of the maze environment, or several orientation-specific maps, and we had hoped that our place cell recordings would help to answer this question. The two learners we obtained place cell recordings from showed no evidence of having place fields specific to the direction in which they were released in the maze. However, these animals failed to exhibit unambiguous early learning from three sides, so these animals appeared to be "eureka" learners, and we would not have expected to observe evidence of piecemeal mapping anyway. On the other hand, it is possible that all of our learners put their maps together in some sort of piecemeal fashion, and yet, ultimately, all ended up with a single unified map. Further study will be required to resolve this issue.

4.7 Reliable drift in the head direction system

As mentioned previously, the HD cell that we recorded from did maintain a stable preferred firing direction inside the curtain across recorded trials. We believe the animal's sense of direction may have been affected by the presence of the recording wires during the initial recording trials, or the amount of restraint used during passive rotation of the animal. Some support for the latter hypothesis comes from evidence that both place cells (Foster et al., 1989), and HD cells (Knierim et al., 1995) tend to become silent when animals are restrained to the degree that movement is extremely difficult. This suggests that, under such conditions, the animal may not be updating it's representations of

direction and place. While the cell in our experiment did not stop firing, it may be that during brief periods of increased restraint, such as when the animal was initially manipulated into position for passive rotation, the rat was not registering changes in its forward facing direction, so that when normal direction tracking resumed, the animal's internal sense of direction was changed.

Unfortunately, the most interesting observations we made regarding the drift in the preferred firing direction of the HD unit we succeeded in isolating could not be quantified for analysis. However, we were sufficiently convinced of the reliability of the observed effect that we feel we can draw some conclusions about how this particular animal's sense of direction changed over the time course of a normal trial. It was clear from our observations, that as predicted by Barlow (1964), internal sense of direction is subject to drift. However, the drift observed in this animal was consistent across trials, much like the shifts observed by Taube and Burton (1995) when animals move into familiar adjacent environments. Even though this rat's internal sense of direction suggested that it had been rotated with respect to the larger environment by the time the rat got to the maze, the amount by which the rat felt it had been rotated was probably less than the 360^0 rotation actually performed. Moreover, because this under-representation of rotation seemed to be cumulative and related to the distance traveled, rather than associated with any distinct map-parsing events, it would have been interesting to see how this animal's field behaved when brought through a closer POE in the curtain. Would, for example, the cell have suddenly shifted its preferred firing direction to the

inside-curtain orientation (southeast), or would it have stayed closer to the starting direction (northwest). Unfortunately, we did not have the opportunity to test this hypothesis, although we would have predicted that the former would not have resulted in an error in choice behaviour, while the latter should have.

4.8 Summary.

This experiment demonstrated that rats trained in dry-land appetitive spatial learning task do not require a stable POE in order to solve the maze problem. These findings are consistent with predictions by Hynes et al. (1998). The animals in this experiment appear to have solved the problem using a map with orientation obtained from the geometry of the experimental room, and maintained by path integration as the animal was carried from the holding rack, through the curtain, and to the maze (in keeping with Gallistel, 1990). The rats were able to use the cue panel to orient their maps on trials when they were intentionally disoriented, but preferred to use their internal sense of direction when they were not. The decision to rely on the cues may depend on whether or not the animal is aware that its internal sense of direction is reliable.

Place fields and behavioural choices responded in a similar manner to our manipulations, suggesting that both are influenced in a similar manner However, on a trial-by-trial basis, the degree of match between the two measures was poor for some animals. Place cell recordings tended to be more reliable across trial than choice behaviour, and our results suggest use of minimum impact probe procedures would be

advisable for future investigations combining choice behaviour and place cell recording. The presence of a stable field in a non-learner further emphasises the importance of eliminating response biases before drawing conclusions from choice behaviour.

In the process of learning the maze problem, there is evidence that some rats constructed their maps in a piecemeal fashion, perhaps initially using individual maps specific to a particular perceived starting orientation and/or local view. Learning to maintain a sense of direction (relative to the experimental room) with between-trials reliability, regardless of its accuracy with respect to the world, may be essential for solution of the maze problem and integration of multiple maps of the same environment. This process may be hindered by experimental procedures which produce unintentional disorientation. Further experimentation might involve the use of behavioural probes and cell recordings at regular intervals during the learning process, before animals have reached criterion, to better determine the nature of the maps animals use before they have acquired a solution to the spatial problem.

Further investigation of the effects of explicit versus subtle disorientation on rats' use of cues for orientation is also recommended. Specifically, it would be interesting to determine whether rotation below the vestibular threshold would lead to the type of reliance on cues that we observed. Recently, Jeffrey et al.(1997) used slow rotation below the vestibular threshold to make controlled changes to rats' internal sense of direction, and found that such rotation almost always resulted in a corresponding rotation in the position of place fields when the animal was replaced in the box it had been trained in.

We predict that in the current experimental paradigm, this type of disorientation, which the animal is unaware of, would have led to predictable patterns of choice and place field rotation that depended on the amount of rotation, but not the position of the cue.

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Table 1

Correlations between rotated versions of the baseline firing rate map and firing rate maps from subsequent trials for subject 15. On trials B, C and D, the cue was rotated 180°. On trial D, the animal was also disoriented. Note that on this trial, the field is most highly correlated with the 180° rotated version of the baseline field.

Record	Pearson's Correlation Coefficients			
A (rotation)	0°	90°	180°	270°
B	**.8722****	-.0298	-.3382**	-.1599
C	**.7734****	.0811	-.3097**	-.2254*
D	-.3044**	-.1990*	**.7950****	.0514

Rotations of the baseline recording (Record A) are considered to be clockwise
Significant positive correlations appear in bold. * $p<.05$ ** $p<.01$

Table 2

Correlations between rotated versions of the baseline firing rate map and firing rate maps from subsequent trials for subject 9. Record B was taken on a 45^0 maze-rotation trial, while record C was taken on a trial in which the curtain was drawn back from around the maze. Compare with Figure 12, and note that no significant correlations result from either 45^0 field rotation or novel field formation.

Record	Pearson's Correlation Coefficients			
A (rotation)	0^0	90^0	180^0	270^0
B	.0152	.0322	-.0914	.0103
C	-.0963	-.0497	.1207	.0172

Rotations of the baseline recording (Record A) are considered to be clockwise.
* $p<.05$ ** $p<.01$

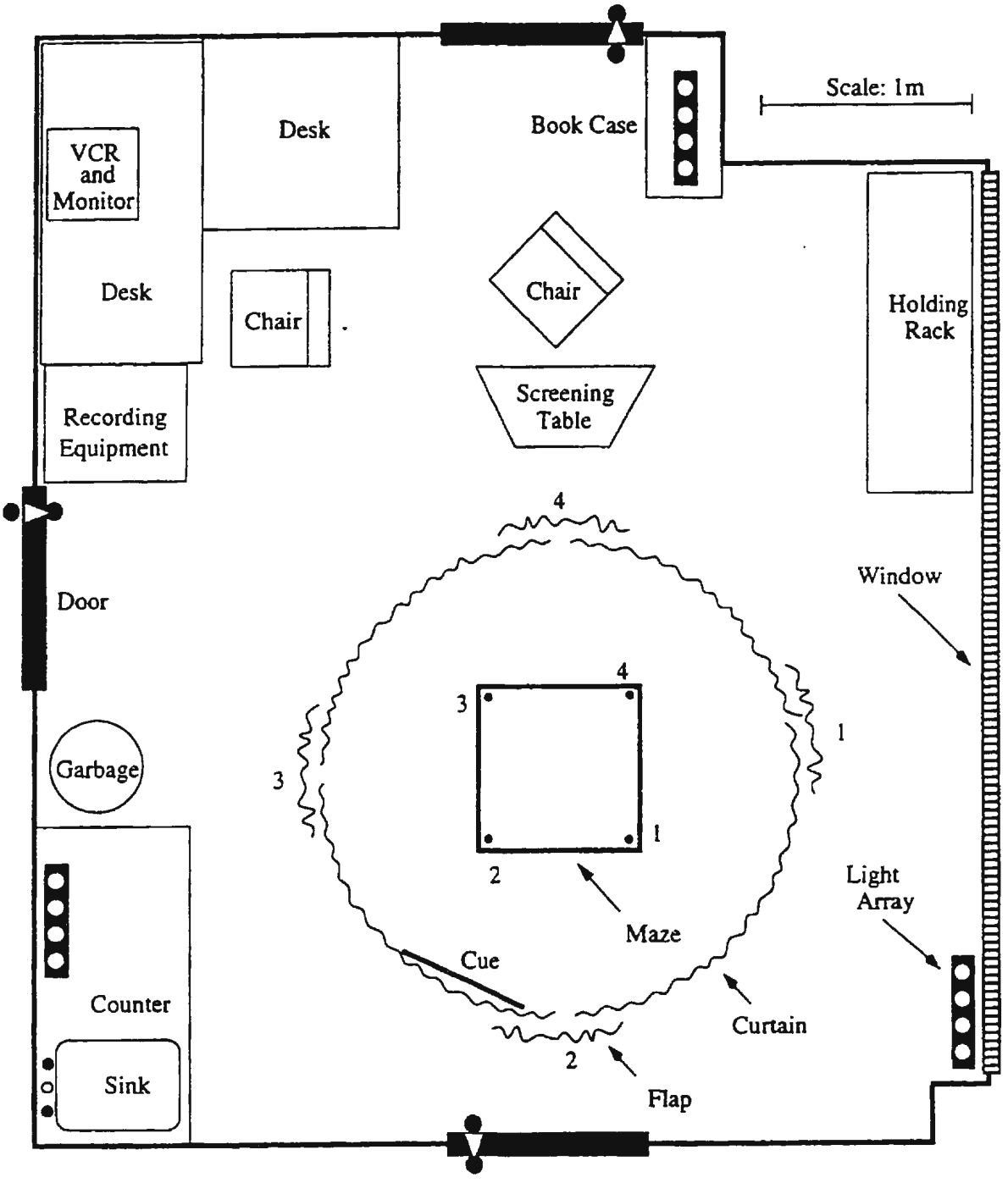
Table 3

Correlations between rotated versions of the baseline firing rate map and firing rate maps from subsequent trials for subject 13. Records B-F were all taken under normal cue conditions, and without disorientation.

Record	Pearson's Correlation Coefficients			
A (rotation)	0^0	90^0	180^0	270^0
B	**.6234****	-.1445	-.2543*	-.1372
C	**.5832****	-.2648**	-.1838	-.1700
D	**.4642****	.0955	-.1987*	-.2398*
E	**.5065****	-.1571	-.2976**	-.1535
F	**.6026****	-.0613	-.3733**	-.165

Rotations of the baseline recording (Record A) are considered to be clockwise
Significant positive correlations appear in bold. *p<.05 ** p<.01

Figure 1. The experimental room. Numbers outside the curtain refer to the number assigned to the adjacent point of entry. Numbers next to the maze indicate the numbering of the corners and sides. The screening table was only in the position indicated during recording sessions: at all other times it was positioned by the wall between the bookcase and the holding rack. The arrows in doors indicate the direction in which they open.


Scale: 1m
Book Case
Desk
VCR and Monitor
Desk
Chair
Chair
Holding Rack
Screening Table
Recording Equipment
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Window
4
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1
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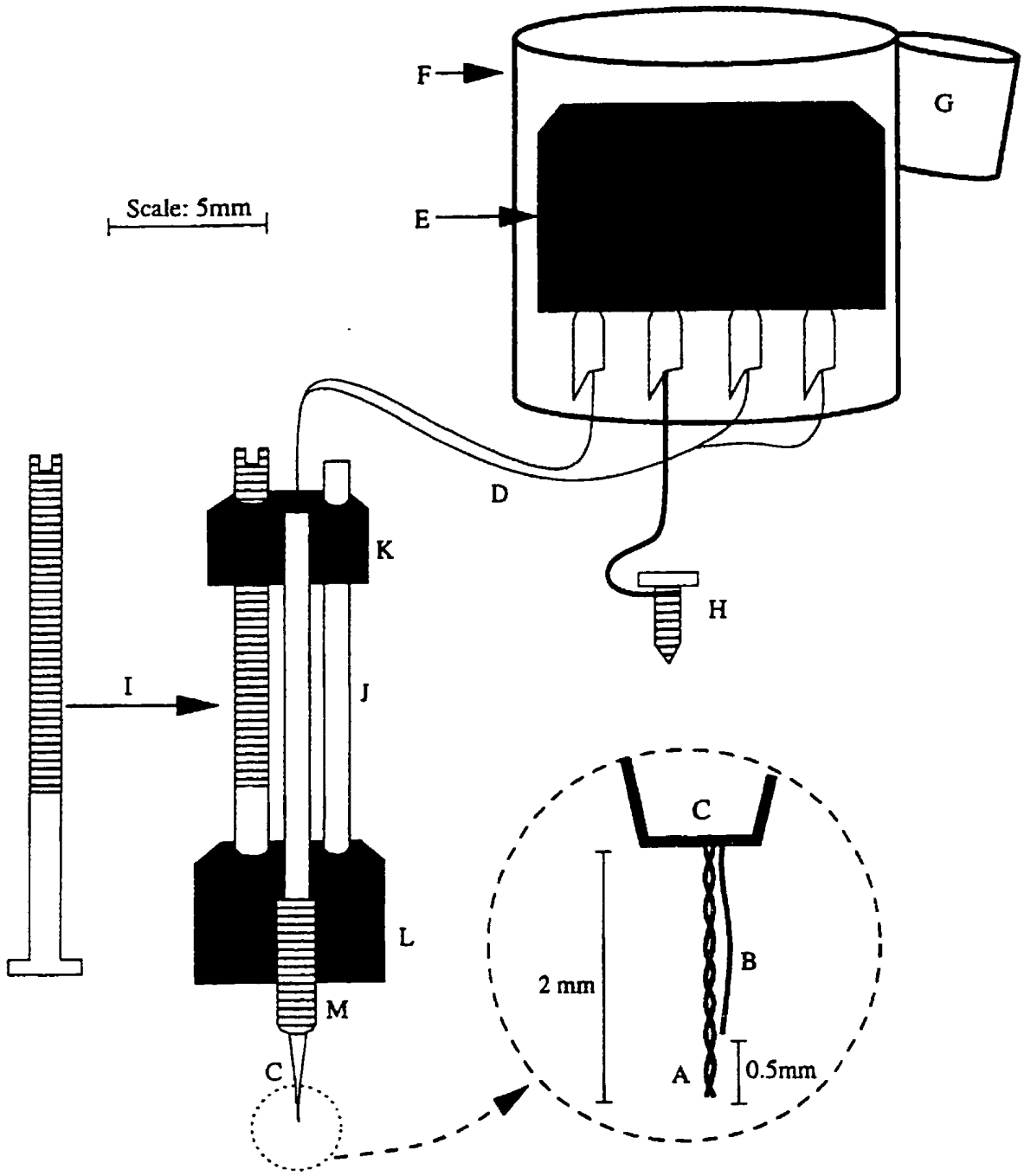
Garbage
Maze
Light Array
Cue
Curtain
Counter
Sink
Flap

Figure 2. The electrode and microdrive. A: twisted-wire stereotrode. B: single-wire reference electrode. C: glass pipette. D: stereotrode and reference electrode wires. E: FET socket. F: microdrive shield. G: FET anchor. H: ground wire and skull screw. I: machined screw used in microdrive. J: guide post of microdrive. K: microdrive top stage. L: microdrive base. M: electrode shield.


F
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Scale: 5mm
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C
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A
0.5mm

Figure 3. Sample paths on training trials. Dotted lines indicate examples of paths taken by the experimenter during training trials, while carrying rats from holding rack to the maze. Each path consists of three components: rack to curtain, curtain to maze, and final rotation to face a randomly selected side. Panels A, B, and C are representative paths used for a random point of entry subject, while D, E, and F represent a fixed point of entry rat who's point of entry is side 2. The experimenter always stands at the side of the maze adjacent to the point of entry for random point of entry animals, while the side the experimenter stands at is varied across trials for fixed point of entry animals.

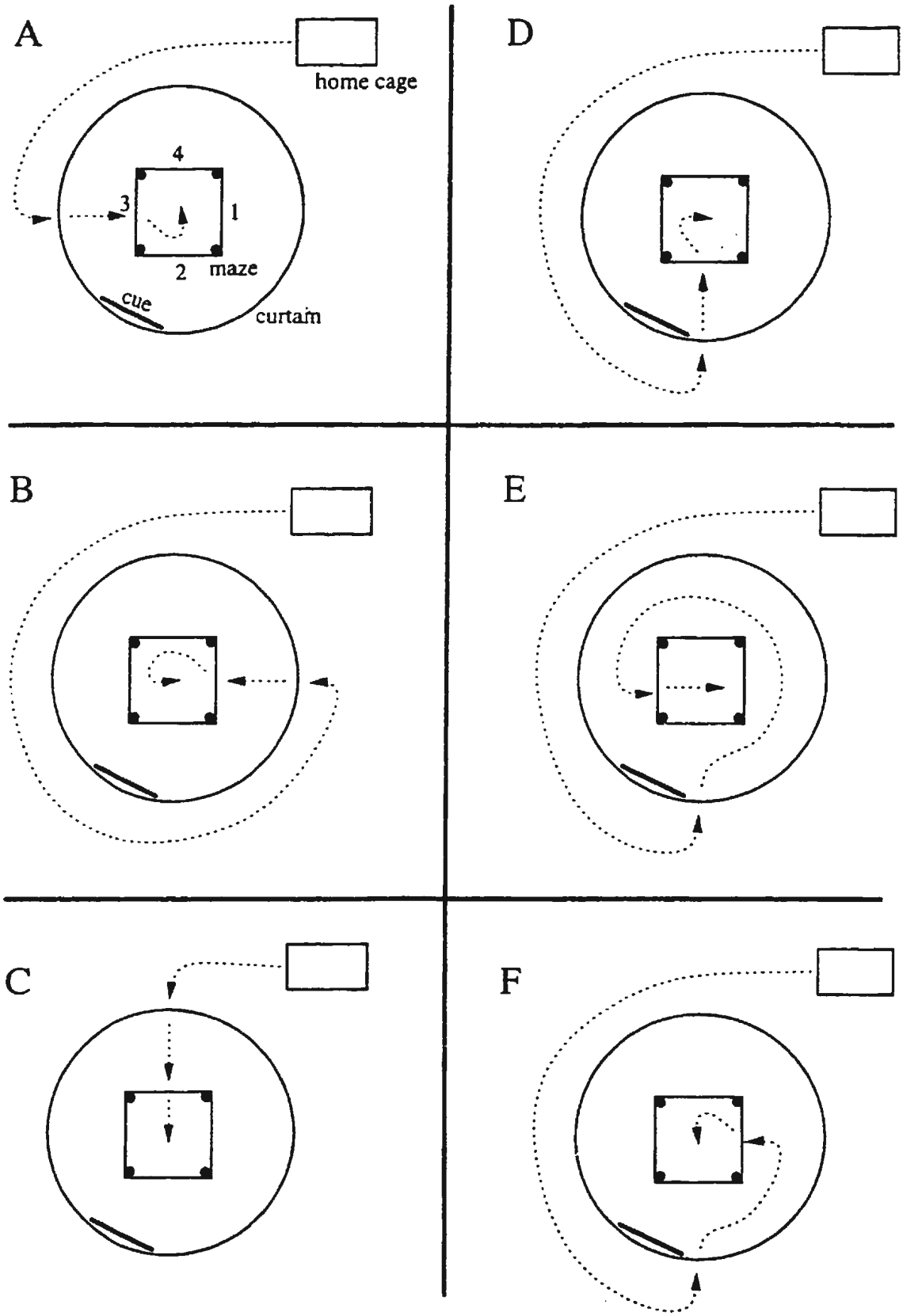
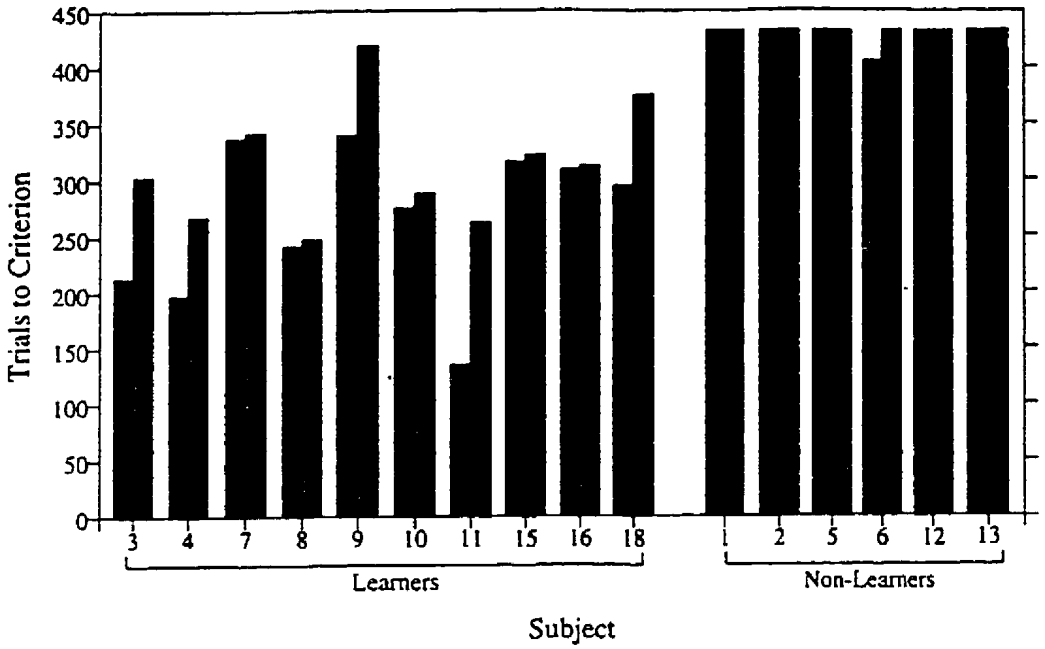
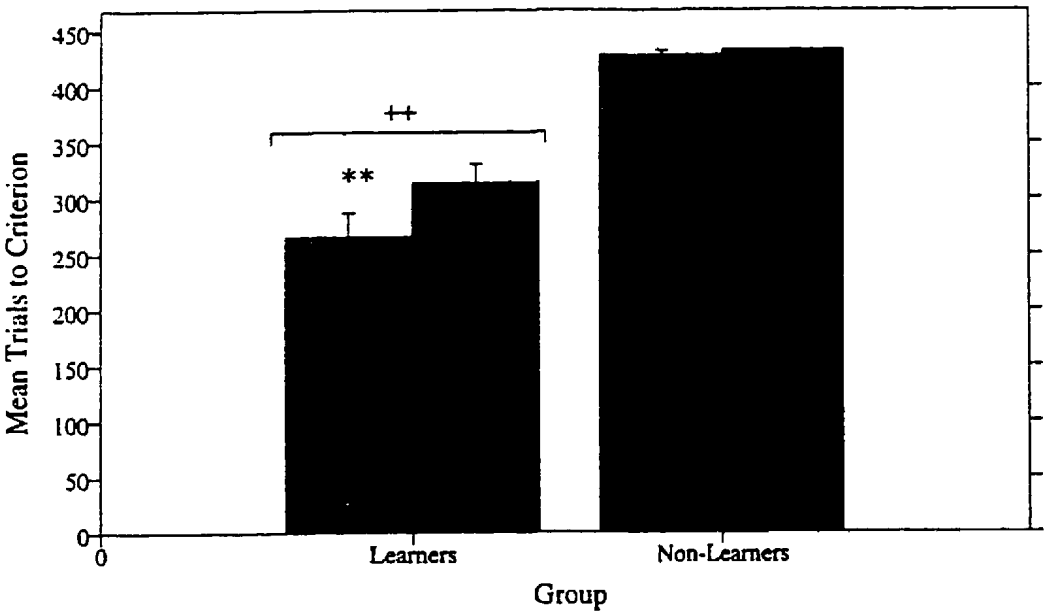

A
home cage
4
3
1
2
maze
cue
curtain
D
B
E
C
F

Figure 4. Trials to criterion for learners vs. non-learners. The top panel shows trials to criterion for each subject, while the bottom compares non-learners with learners. In both panels, grey bars represent overall trials to criterion, while black bars indicate the earliest trial on which criterion would have been reached if we ignore trials on which animals are released facing in a direction from which they made the most errors. ** and ++ indicate differences that are significant at the $p<.01$ level.



Trials to criterion from any 3 sides

Trials to criterion from all 4 sides



* - ■ different from ■

+ - Learners differ from non-learners

Figure 5. Four patterns of task acquisition. Each panel shows the performance of an animals over the course of training, broken down by the side the animal was facing upon release on each trial. Each block represents data collapsed across three recording days. Subject 4 acquired a solution from all four sides at about the same time, and might be what we refer to as a "eureka" learner. Subject 3 learned from three sides first, and subject 16 learned from two before reaching overall criterion. Subject 5 is a non-learner who refuses to turn around and randomly chooses between going forward and turning right or left. As a result, this rat has a 50% chance of making a correct choice when facing two sides of the maze, and a 0% chance facing the other two.

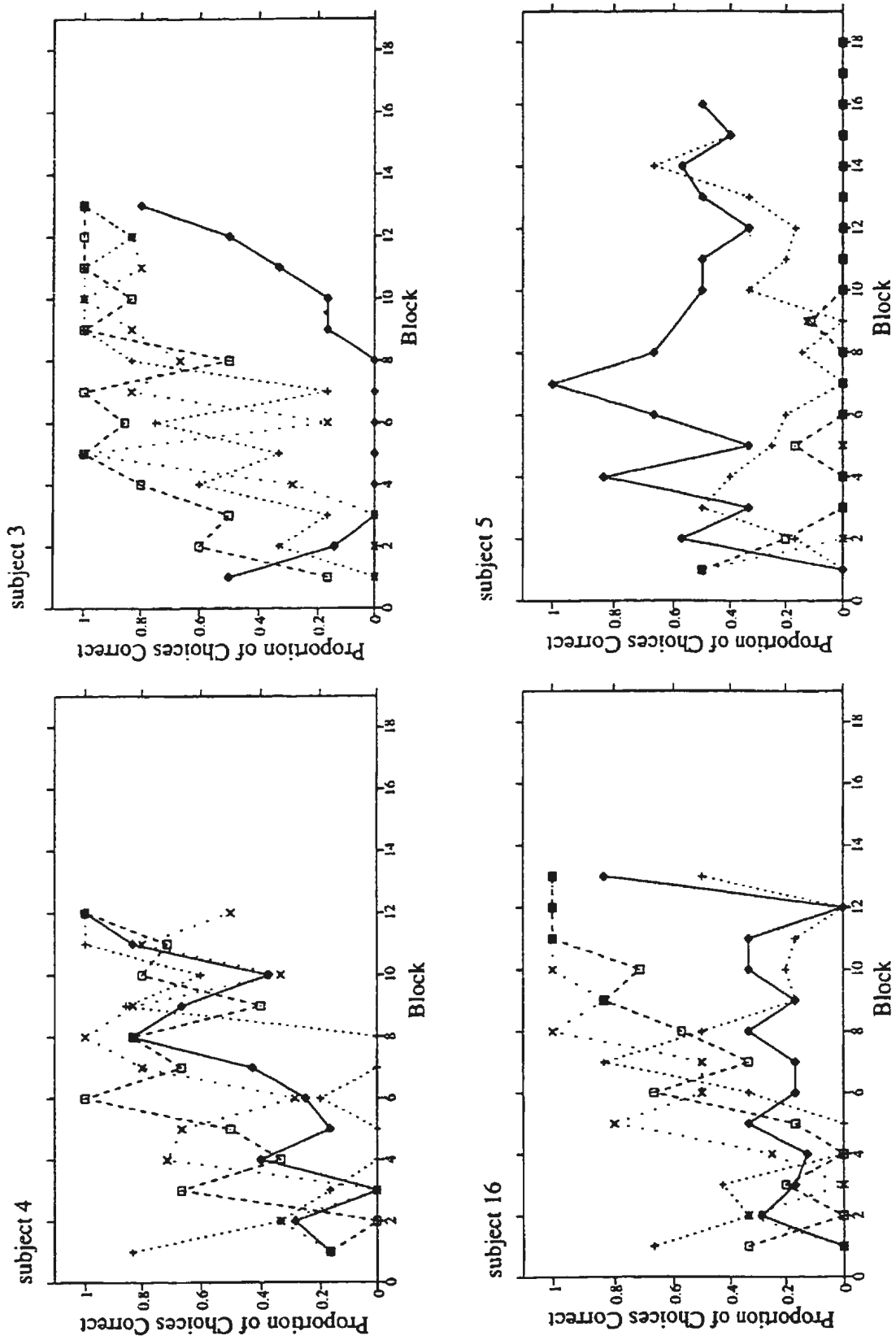

subject 3
subject 4
subject 5
subject 16
Proportion of Choices Correct
Block

Figure 6. The effect of incidental rotation prior to release. Training trials on which animals were released facing either away from (0^0), to the left of (90^0), to the right of (90^0) or directly towards (180^0) the experimenter were combined to compare the number correct choices that were made under each condition. * indicates a significant difference at the $p<.05$ level.

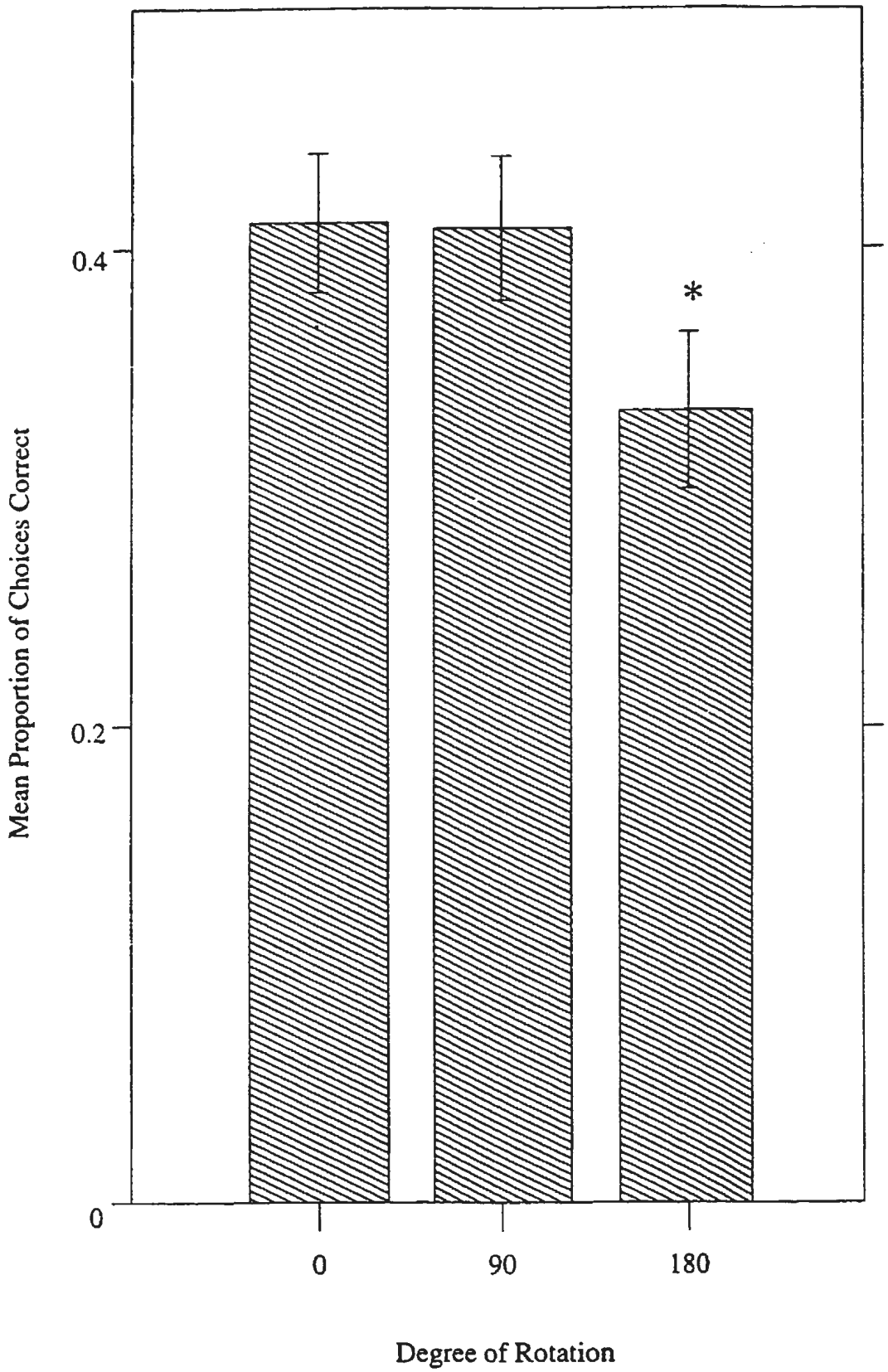

Mean Proportion of Choices Correct
0.4
0.2
0
*
0
90
180
Degree of Rotation

Figure 7. The effect of behavioural probes. For each type of probe, proportion of correct choices on probe trials (dark grey), rotationally-correct choices on probe trials (light grey), and correct choices on control trials (black) are illustrated. * and + indicate a significant difference at the $p<.05$ level. ** and ++ indicate a significant difference at the $p<.01$ level.

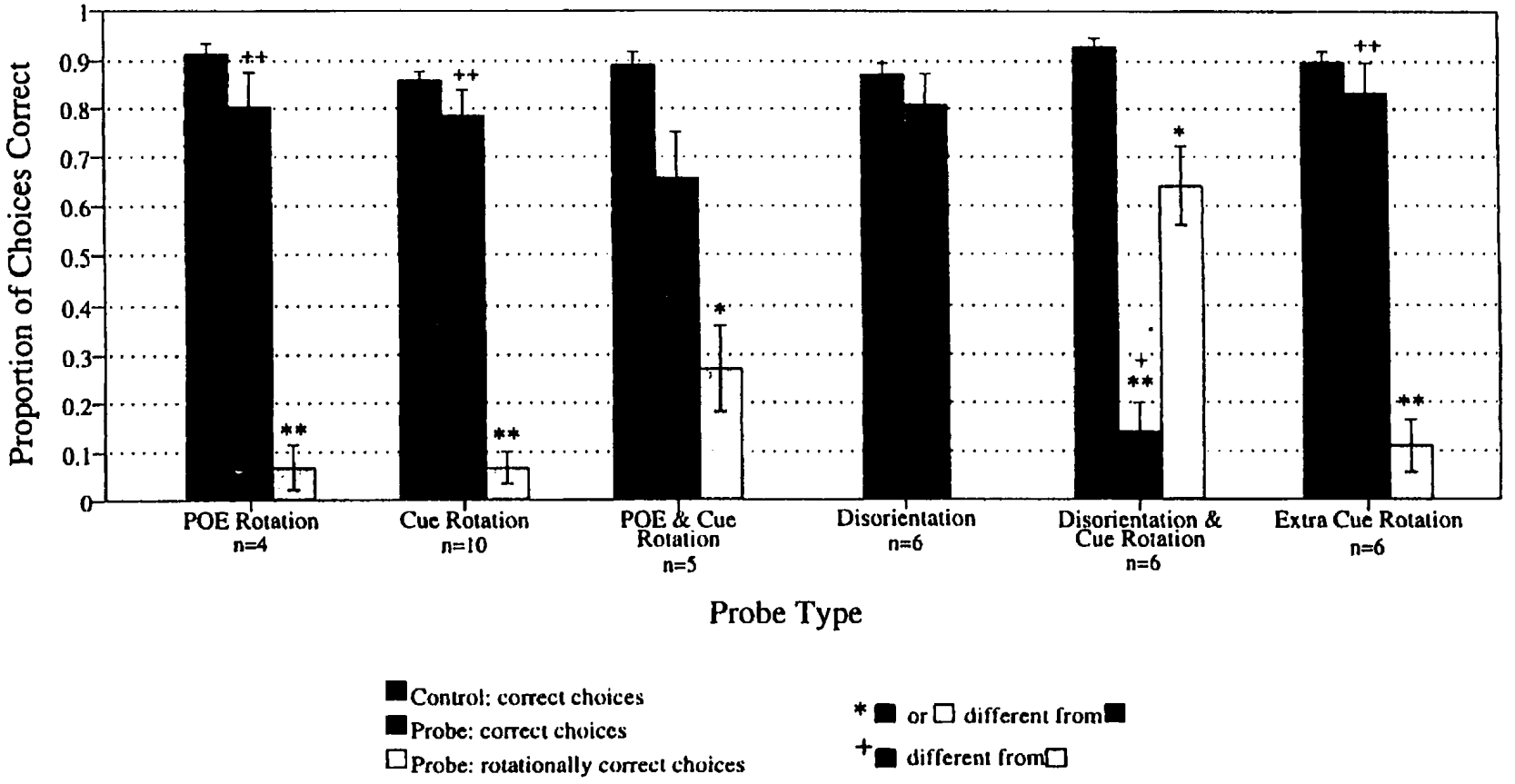
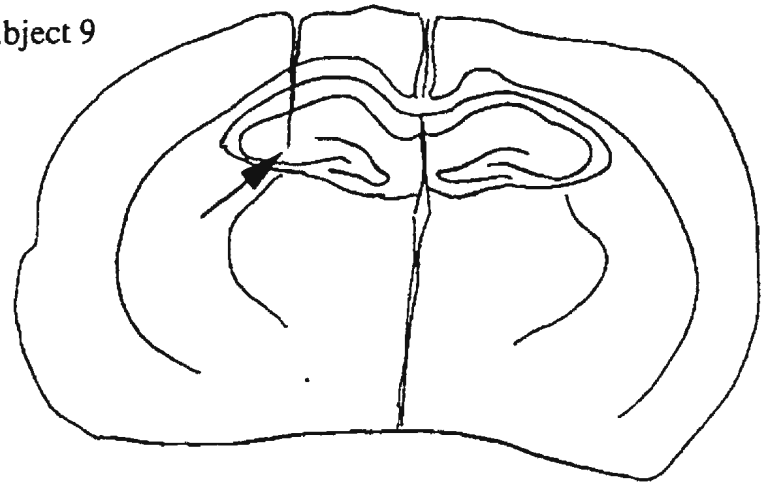

Proportion of Choices Correct
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
++
**
++
**
*
**
+
*
++
**
POE Rotation
n=4
Cue Rotation
n=10
POE & Cue
Rotation
n=5
Disorientation
n=6
Disorientation &
Cue Rotation
n=6
Extra Cue Rotation
n=6
Probe Type
Control: correct choices
Probe: correct choices
Probe: rotationally correct choices
* or different from
+ different from

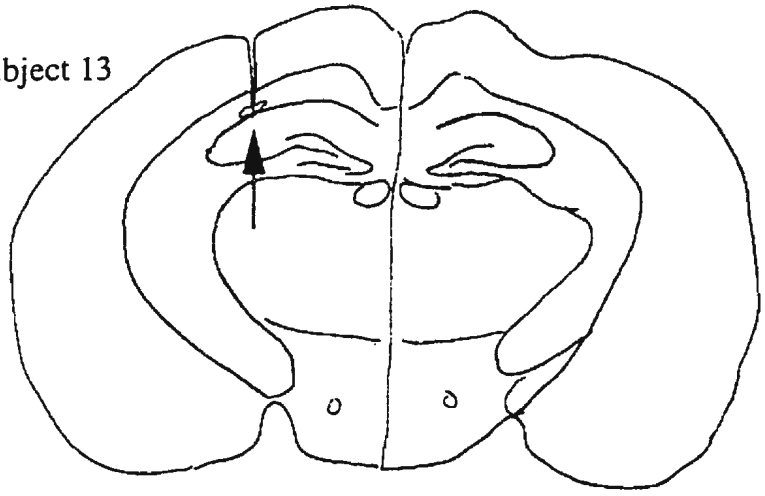
Figure 8. Histology. Drawings of electrode tracks in 25 μm coronal Nissl stained sections. Each panel shows how the electrode passed through the CA1 region of the hippocampus in all three animals from whom place cell recordings were obtained. Large arrows indicate the position of the tip of the electrode in each section.

Subject 9



Scale:
5mm

Subject 13



Subject 15

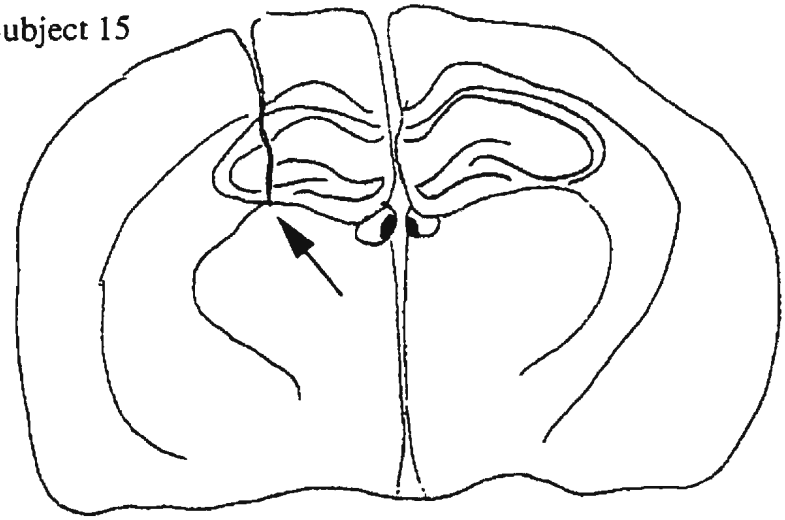


Figure 9. Representative waveforms from recording sessions. Top traces in each panel are from stereotrode channel-2. Bottom traces are from channel-1. Note that scale varies from panel to panel. A: head direction cell from subject 9. B: place cell from subject 9. C: the same cell, but exhibiting a novel field. D: a different place cell from subject 9. E: A place cell from subject 13. F-G: two different place cells from subject 15.

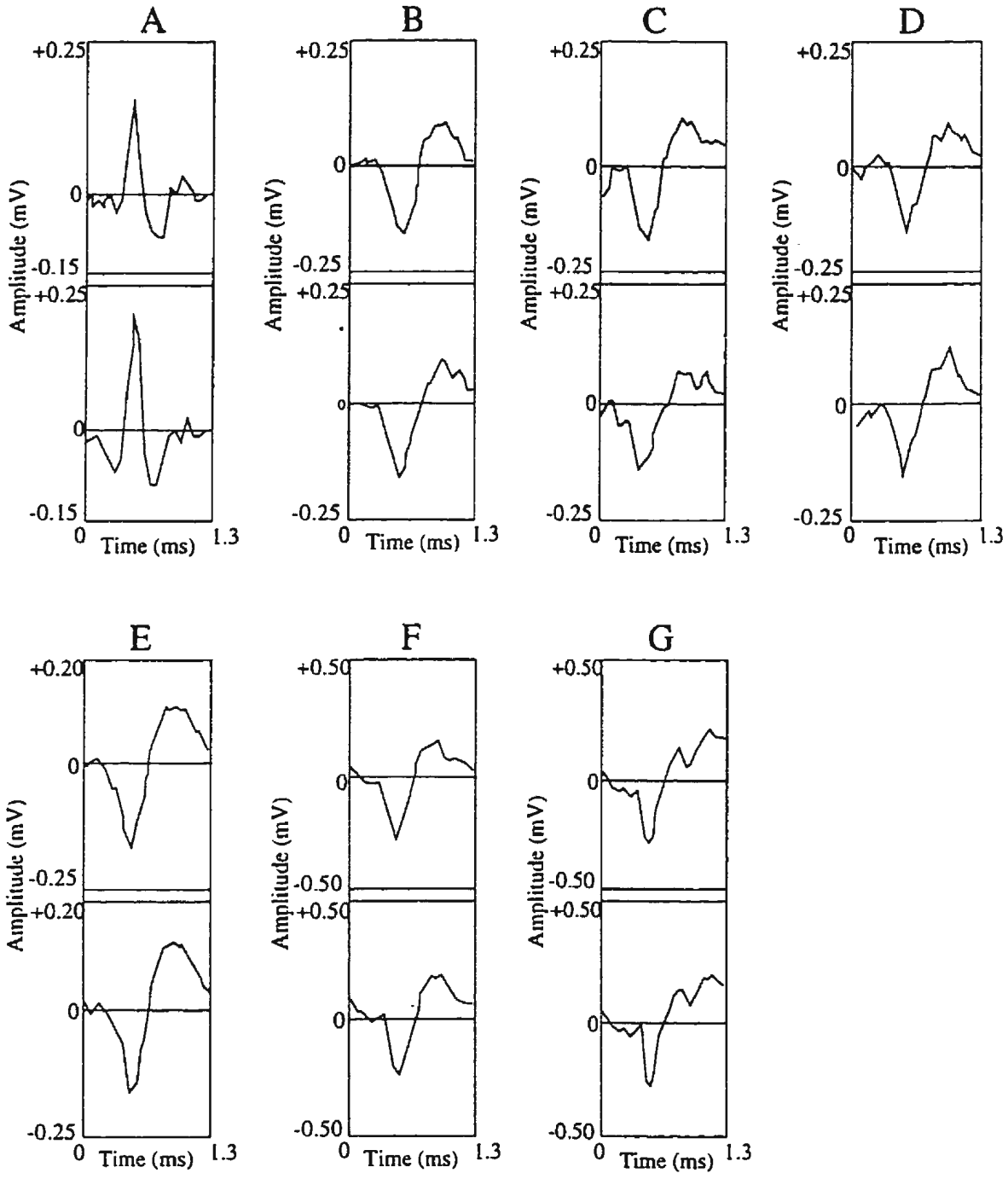

A
B
C
D
E
F
G
Amplitude (mV)
Time (ms)
+0.25
0
-0.15
-0.25
+0.20
-0.20
+0.50
-0.50
1.3

Figure 10. The effects of probes on place fields. Fields can respond in one of three ways on any recording trial - by staying in the normal position (black), by rotating in accordance with the manipulation (dark grey), or by responding in an unpredictable manner (light grey). Combined, the three choice types sum to 1 for each type of probe. As shown, cue rotation leads to little disturbance of place field position, while disorientation leads to a considerable amount of cue tracking when the cue is rotated.

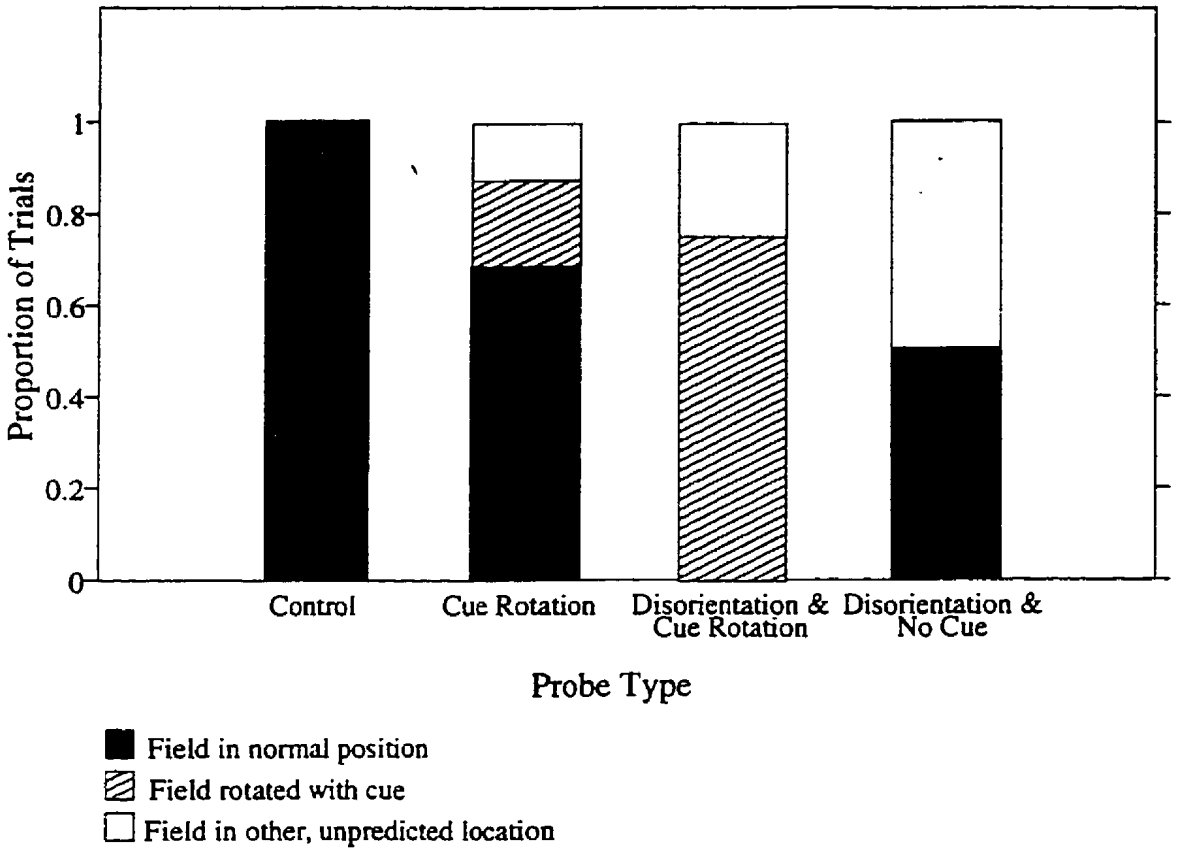

Proportion of Trials
1
0.8
0.6
0.4
0.2
0
Control
Cue Rotation
Disorientation & Cue Rotation
Disorientation & No Cue
Probe Type
Field in normal position
Field rotated with cue
Field in other, unpredicted location

Figure 11. An example of a stable field undergoing rotation. Row 1 shows the positions in the maze that the animal visited, as viewed by the overhead camera. Row 2 shows the positions in the maze where the place cell fired. Row 3 shows the firing rate of the cell in each bin in a 10 by 10 matrix representing the maze. Note that the plots in rows 2 and 3 are scaled to the maze box itself, while row 1 includes spaces the animal could not have visited, but which were registered by the video camera. The rate for each bin is calculated by dividing the number of spikes that occur in each bin by the dwell time in that bin. Firing rates are illustrated as a percentage of the peak firing rate for that cell, colour coded with black representing 0-10% and white representing 100% - that is, white blocks are the bins where the cell fired maximally, and usually represent the center of the cell's place field. Columns A, B, C, and D represent four consecutive recording sessions from subject 15. A is the baseline session, in which the field appears in the lower right hand corner. B and C illustrate trials in which the field remained in the same corner, despite the fact that the cue had been rotated by 180^0. Column D represents a trial on which the cue was rotated 180^0 and the animal was disoriented. Clearly, the field has also rotated by 180^0, to the top left corner. Referring to the plot of position samples shows that bins with very low firing rates are not simply the result of a failure to spend sufficient time in those bins.

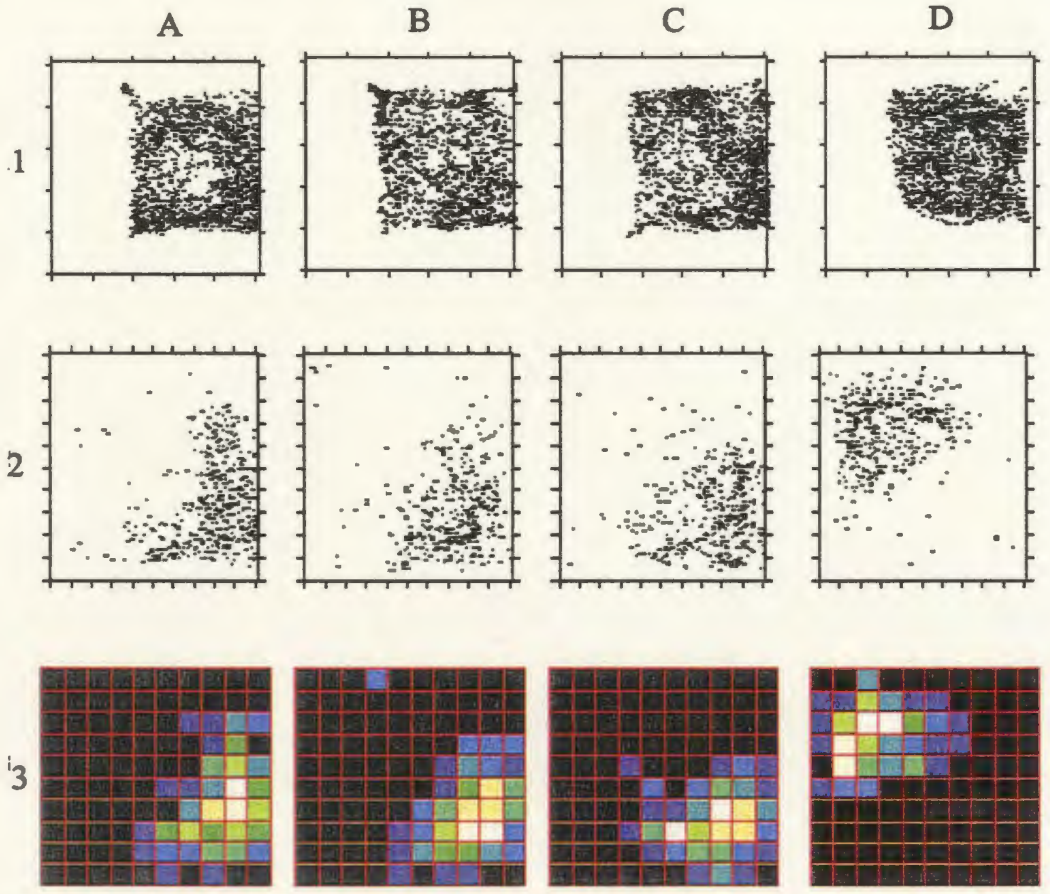

A
B
C
D
1
2
3

Figure 12. An example of box-geometry tracking and remapping. Rows 1, 2 and 3 represent data as described in Figure 10. Columns A, B and C represent three consecutive recordings from subject 9. Column A is the baseline recording for that day, and illustrates a distinctive, J-shaped field with peak firing near the center of the maze.. Column B represents a trial on which the maze was rotated by 45^0. This is evident from the position sampling illustrated in row 1. In order to demonstrate how the amount of cell firing and, accordingly, the place field, have remained linked to the geometry of the maze, the actual position of the maze has been overlaid on rows 2 and 3. Clearly, the field has rotated 45^0 counter-clockwise. Column C represents a trial on which the curtain was removed, giving the rat visual access to the entire experimental room. The distinctive shape of the field disappeared, and the peak firing rate shifted away from the center of the maze, towards the side. This suggests that on this trial, the animal was treating the maze as a different environment, not a rotated version of the familiar one.

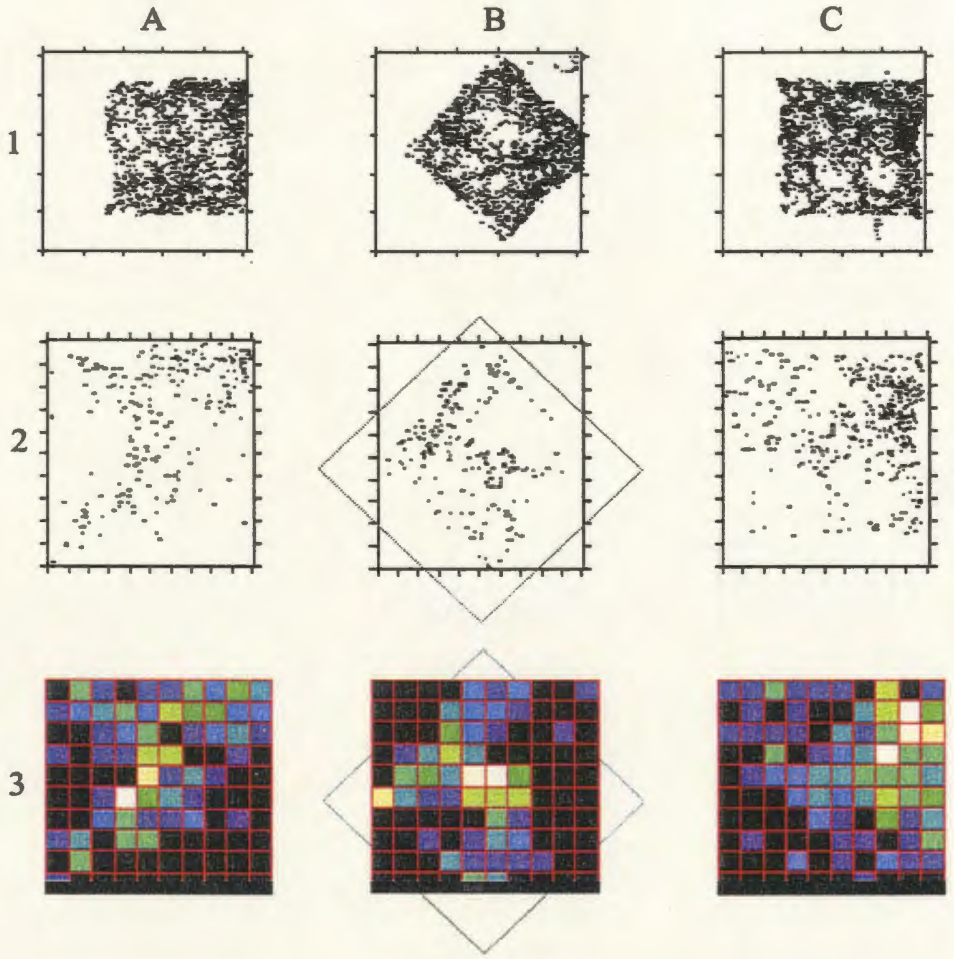

A
B
C
1
2
3

Figure 13. A stable place field in a non-learner. Columns A-F represent six consecutive records taken from subject 13 under normal trial conditions for that animal. Without reference to the dwell time in each bin, simple observation of where the cell fired (rows 1 and 3) do not suggest any particular region of peak firing. However, when dwell time is taken into consideration, it is clear that the field is well defined and anchored to the lower left side of the maze on all six trials.

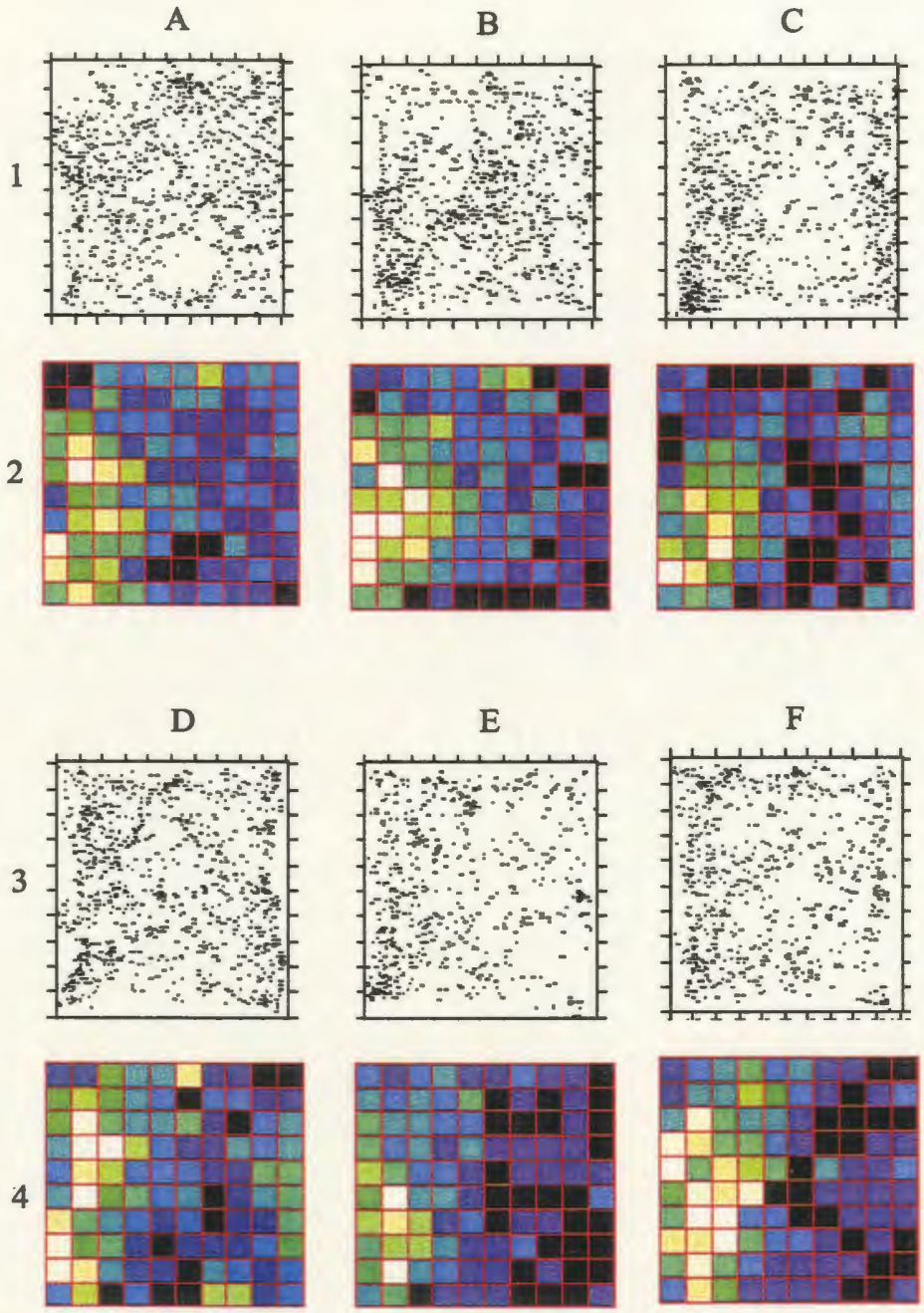

A
B
C
1
2
D
E
F
3
4

Figure 14. Unstable responses from a head direction cell. Recordings were obtained from a single cell in subject 9. The animal was passively rotated through 180^0, while 5 second recordings were taken with the animal's head facing N, NE, E, SE, S, SW, W, and NW. Panels A-G are polar coordinate plots of percentage-of-peak (POP) firing rate for the cell while the animal faces in each direction. Peak rates for a given trial are shown under each plot. Dark solid lines connect eight points representing the POP firing rate with the animal facing in each direction. Excursion of each point from the origin represents the POP firing rate. The four concentric circles in each plot (dotted lines) indicate 25%, 50%, 75% and 100% of the peak firing rate. While on the screening table, the cell always fired maximally when the animal was facing north, as illustrated in panel A. Panels B and C show how the cell shifted preferred firing direction even under normal conditions, and continued to do so, although not in any consistent or predictable manner, when the cue was rotated by 90^0 clockwise (panels D and E) and by 180^0 (panels F and G).

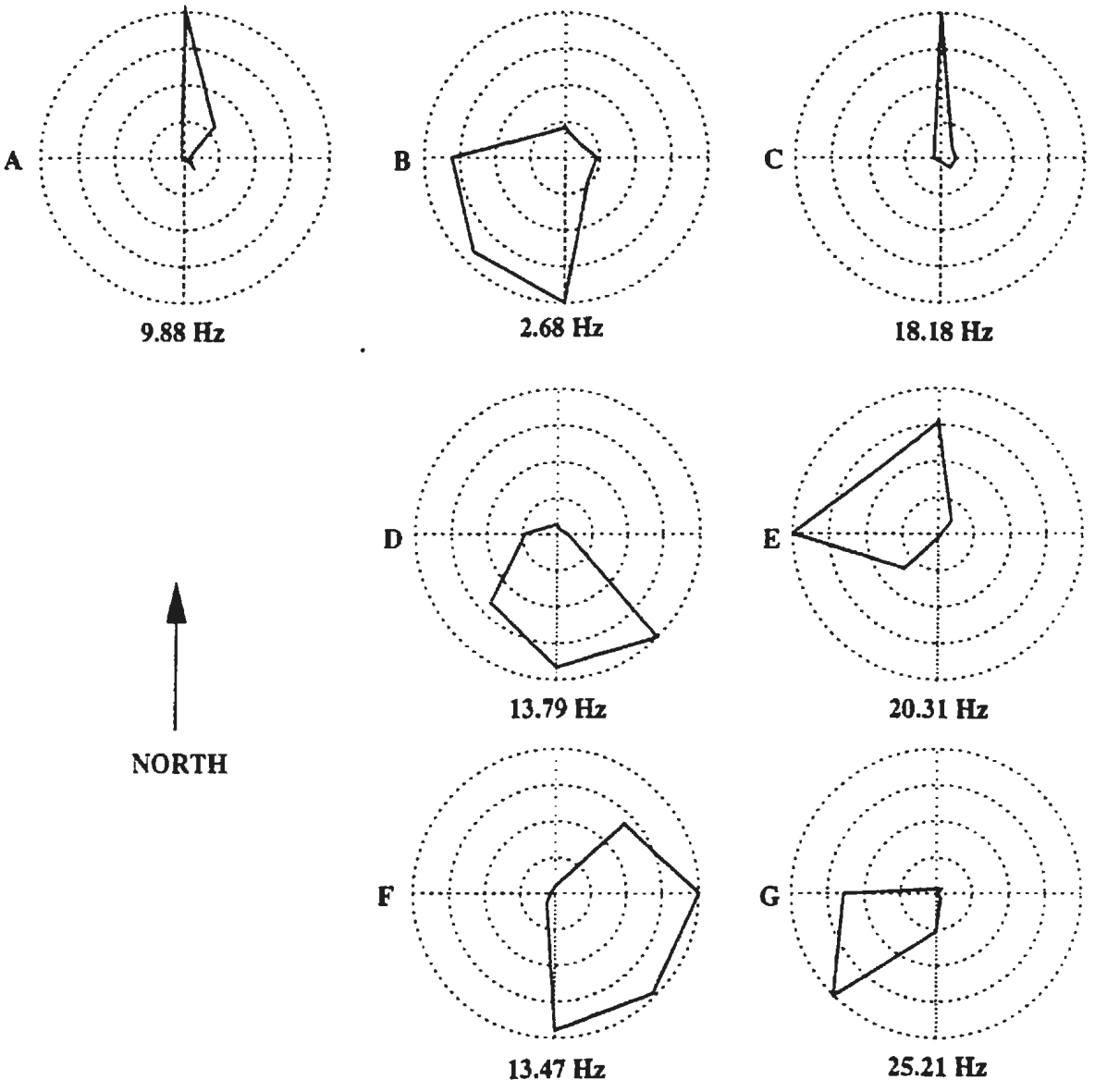

A
9.88 Hz
B
2.68 Hz
C
18.18 Hz
D
13.79 Hz
E
20.31 Hz
NORTH
F
13.47 Hz
G
25.21 Hz

Figure 15. Drift of head direction cell tuning. The dotted line indicates the path the experimenter used in transporting subject 9 from the screening table to the maze on five consecutive trials. On each trial, and at regular intervals along the route, the animal was passively rotated by 360^0 several times, slowly and in both directions. Large black arrows indicate the preferred firing direction of the head direction cell at each of 6 sampling positions. Given that the animal was undergoing unintentional counter-clockwise rotation as the experimenter walked counter-clockwise around the curtain to the point of entry, the arrows may be taken as an indication of how far in a clockwise direction the animal believes it must turn in order to be facing north again. The pattern of underestimation of the amount by which the animal was actually being rotated en route to the maze was consistent across trials. On the trip back to the screening table, preferred firing direction seemed to remain locked to the southwest direction once the animal exited through the curtain, and remained that way until the animal had been on the screening table for a few seconds, after which the preferred firing direction became north again.

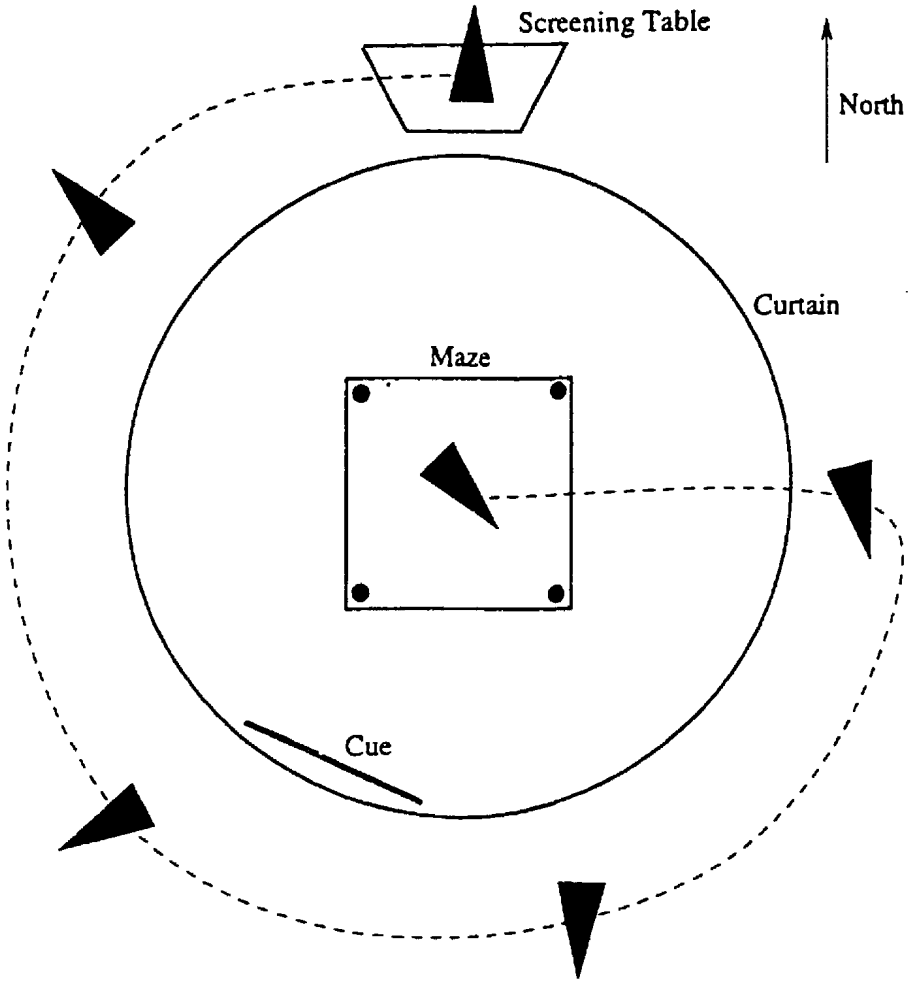
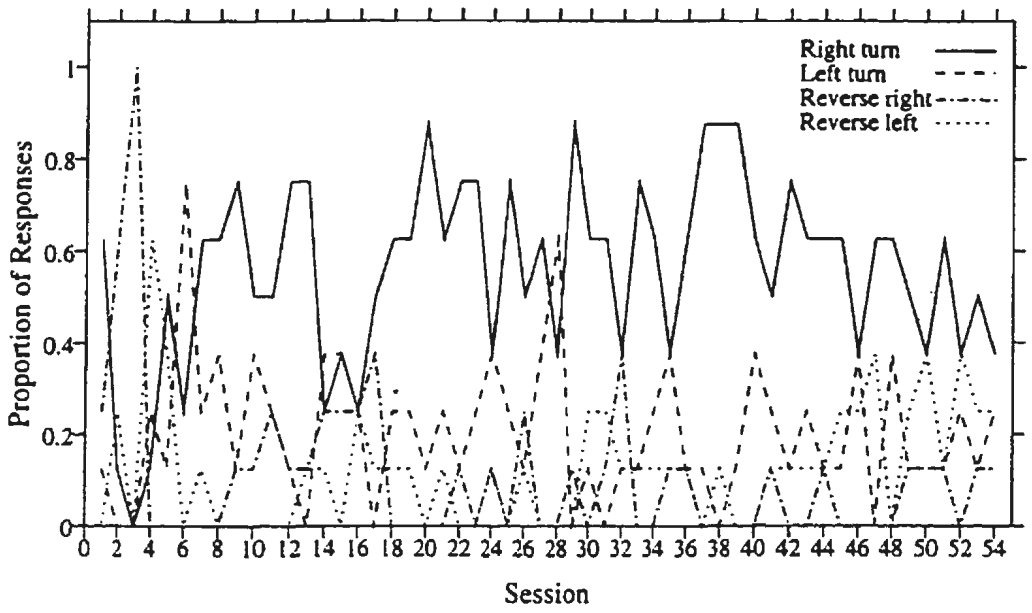

Screening Table
North
Curtain
Maze
Cue

Figure 16. Response bias in subject 13. Each line plots the proportion of the rat's choices which were of a particular type across training days (sessions). Left and right refer to the rat running straight ahead upon release, and choosing the corner either to its left or right, respectively. Reverse left and reverse right refer to the rat choosing the corner behind it and to the left (reverse left) or right (reverse right). Subject 13 consistently tends to run straight ahead and to the right throughout most of the training phase.


Right turn
Left turn
Reverse right
Reverse left
Proportion of Responses
0
0.2
0.4
0.6
0.8
1
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54
Session

Appendix A
Place Field Construction Program

```
#! /bin/sh
nawk '

BEGIN {
printf("\n\n\nPLACE CELL ANALYSIS PROGRAM  by J Huxter, 1998")
while(choice!="q")              {
print"\n*****************************************************************
print"MAIN MENU  file = \""origfile"\", x-bins="xbintot", y-bins="ybintot

printf("Choice ([o]ptions) ? "), getline $0 < "/dev/tty"
if($1=="1") PLACE1(origfile,basefile)
if($1=="2") PLACE2(basefile,xbintot,ybintot)
if($1=="p1") PLOT1(basefile)
if($1=="p2") PLOT2(basefile)
if($1=="p3") PLOT3(basefile,xbintot,ybintot)
if($1=="f") FILE()
if($1=="c") CLUSTERS(basefile)
if($1=="b") BINS()
if($1=="r") ROTATE(basefile)
if($1=="s") SPLIT(basefile)
if($1=="o") OPTIONS()
if($1=="ls") LS()
if($1=="q") QUIT()
                                        }
exit
}


function PLACE1(origfile,basefile) {
#############################################################
# eliminate commas, control-characters from discovery file
# convert numeric cluster ids to numbers (A -> 11, B -> 12)
# convert time-stamp into seconds
# calculate max/min xy coordinates
#               infile = file to read data from with following format
#                               "P",timestamp,x,y
#                               "E"or"S",timestamp,cluster,electrode channel
#
#               x = x coordinate from infile
#               y = y coordinate from infile
#               c = cluster number from infile
#               t = timestamp (converted to seconds from start) from infile
#               tmin = time at start of record (in ms)
#               xmin/xmax = min and max x-coordinates sampled
#               ymin/ymax = min and max y-coordinates sampled
#               tmin = time stamp at start of record
#               tpos = total number of position samples
#               ttime = time at end of record (in seconds, from start)
#               tspk = total number of spike events
#               sx[]/sy[]/st[] = x-coord/y-coord/time for each spike
#               px[]/py[]/pt[] = x-coord/y-coord/time for each position sample
#               clot[] = total number of spikes from each cluster
#
# output =      * sta (statistics)
#                               * spk (position & spike data)
#############################################################
print"\n   CONVERT INPUT FILE & GENERATE STATS"
print"   ----------------------------------"
if(origfile=="") {print"{ERROR}. set input file first!",return}
infile=origfile
FS=","
z=tpos=tspk=ttime=tmin=0
xmin=ymin=999999;xmax=ymax=0
print "   * reading \""infile"\""
print "   * converting timestamp, adding xy data to spikes"
while((getline $0 < infile) > 0)                {
 if($1=="B") continue
               z++
               if(z==1) tmin=$2
               t=($2-tmin)/10000
 if($1=="P")   {
```

```
                        tpos++
                        x=$3,y=$4
                        if(xmin>=x && x!=0) xmin=x; if(xmax<=x) xmax=x
                        if(ymin>=y && x!=0) ymin=y; if(ymax<=y) ymax=y
                        px[tpos]=x;py[tpos]=y;pt[tpos]=t
                                }
        if($1=="E" || $1=="S")  {
                        tspk++
                        c=$3; if(c=="A")c="10"; if(c=="B")c="11"
                        sx[tspk]=x;sy[tspk]=y;st[tspk]=t;sc[tspk]=c;ctot[c]++
                                }
                                                                        }
        close infile
        ttime=t
        xrange=xmax-xmin;yrange=ymax-ymin
        print"   * converting xy coordinates to percentages of xy range"

        print"   * working on position data..."
        close outfile
        outfile=basefile".pos"
        for(z=1;z<=tpos;z++)        {
                        x=((px[z]-xmin)/xrange)*100;y=((py[z]-ymin)/yrange)*100
                        printf("%8.3f %7.3f %7.3f\n", pt[z],x,y) > outfile}
        print"   * position data sent to \""outfile"\""

        print"   * working on spike data..."
close outfile
outfile=basefile".spk"
for(z=1;z<=tspk;z++)        {
        x=((sx[z]-xmin)/xrange)*100;y=((sy[z]-ymin)/yrange)*100
        printf("%8.3f %7.3f %7.3f %2s \n", st[z],x,y,sc[z]) > outfile}
print"   * position spike data sent to \""outfile"\""

close outfile
outfile=basefile".sta"
print"   * parameters sent to \""outfile"\"\n"
printf("%4s (xmin) %4s (ymin) %4s (xmax) %4s (ymax) %6s (samples) %6s (spikes) %7.3f (time)\n",
    xmin,ymin,xmax,ymax,tpos,tspk,ttime) > outfile
z=0
for(c=0;c<=11;c++)
        if(ctot[c]!="") {
                        z++;printf("%-2s %2s %4s %5.3f\n",c,z,ctot[c],ctot[c]/t) >outfile
                        }
close outfile
status=status"BCD"
return
}


function PLACE2(basefile,xbintot,ybintot) {
#################################################################
# calculate dwell-time per bin, spikes per bin
# calculate firing rates for each cluster in each bin
#################################################################
print"\n   SORT SPIKE-EVENTS & DWELL-TIME INTO BINS"
print"   ------------------------------"
if(basefile=="") {print"   [ERROR]  set input file first!",return}
if(xbintot=="") {print"   [ERROR]. set x-bins and y-bins first!",return}
match(status,"B")
 if(RSTART<=0) {print"   [ERROR]. stats file ( sta) missing",return}
match(status,"C")
 if(RSTART<=0) {print"   [ERROR]: spike file ( spk) missing",return}
match(status,"D")
 if(RSTART<=0) {print"   [ERROR]: position file (.pos) missing",return}

FS=" "; infile=basefile".spk"; split("",spike), split("",samp)

z=0;infile=basefile".sta"
print"   * reading parameters from \""infile"\""
while((getline $0 < infile) > 0)  { z++
    if(z==1) {xmin=$1,ymin=$3,xmax=$5,ymax=$7,tsamp=$9,tspike=$11,ttime=$13,continue}
    if($1!="")    {c=$1;id[c]=$2;cspike[c]=$3;crate[c]=$4}}
cmax=id[c], close infile

infile=basefile".spk"
print"   * calculating spikes/bin/cluster from \""infile"\""
while((getline $0 < infile) > 0)                {
        if($2<=-0.001) {xbin=ybin=0;spike[xbin,ybin,id[$4]]++;bspike[xbin,ybin]++,continue}
        xbin=int($2/(100.001/xbintot))+1;ybin=int($3/(100.001/ybintot))+1
```

```
                spike[xbin,ybin,id[$4]]++,bspike[xbin,ybin]++}
close infile

infile=basefile"_pos"
print"    * calculating samples/bin from \""infile"\""
while((getline $0 < infile) > 0)                                {
                if($2<=-0.001) {xbin=ybin=0;samp[xbin,ybin]++;continue}
                xbin=int($2/(100.001/xbintot))+1,ybin=int($3/(100.001/ybintot))+1
                samp[xbin,ybin]++}
close infile

print"    * calculating dwell time & firing rates/bin"
close outfile
outfile=basefile"_bin"
for(xbin=0;xbin<=xbintot;xbin++)                                {
for(ybin=0;ybin<=ybintot;ybin++)                                {
if((xbin==0 && ybin==0) || (xbin!=0 && ybin!=0)){ # only process some bins
                if(samp[xbin,ybin]=="") dwell[xbin,ybin]=0
                if(samp[xbin,ybin]!="") dwell[xbin,ybin]=(samp[xbin,ybin]/tsamp)*tsmne

printf("%3s %3s  %7.3f  ", xbin,ybin,dwell[xbin,ybin]) > outfile

for(c=1,c<=cmax;c++)        {
   x=0
   if(dwell[xbin,ybin]!=0) x=spike[xbin,ybin,c]/dwell[xbin,ybin]
   printf(" %6.3f",x) > outfile
                                                }
print"" > outfile
}}}
close outfile
print"    * data sent to \""outfile"\""
print"\n   Cluster   Id   Spikes  Meanrate\n"
for(c=0;c<=12,c++) {
                if(id[c]>="0") printf("    %3s    %4s    %4s %8.3f\n",c,id[c],cspike[c],crate[c])}
status="status"E"
return
}


function PLOT1(basefile)    {
#########################################################################
if(basefile=="") {print"\[ERROR]  set input file first!",return}

FS="."
infile=basefile".txt"
outfile=basefile"_posall.tmp"

print"\n   PLOT ALL POINTS VISITED IN "infile
print"   ------------------------------------------------"
print "    * reading \""infile"\""
while((getline $0 < infile) > 0)                {
 if($1=="P")                    {print $3,$4 > outfile}
                                                                        }
close infile
close outfile

infile=outfile
outfile="plot.tmp"
print "#! \/usr\/local\/bin\/gnuplot\n" > outfile
print "set bar 0.05\nset key\nset bmargin 3\nset lmargin 3\n" > outfile
print "set xlabel \"x-coordinate\" 0,-2" > outfile
print "set ylabel \"y-coordinate\" -2, 0" > outfile
print "set xtics axis mirror 50" > outfile
print "set ytics axis mirror 50" > outfile
print "set xrange [0:255]" > outfile
print "set yrange [0:255]" > outfile
print "set nogrid" > outfile
print "set key outside" > outfile
print "plot \\"   > outfile
print "\""infile"\" using 1:2 with dots"        > outfile

print "    * generating plot "basefile"_posplot.fig"
print "set term fig color" > outfile
print "set output \""basefile"_posplot.fig\"" > outfile
print "plot \\" > outfile
print "\""infile"\" using 1:2 with dots"        > outfile


print "\npause -1 \"* clear plot\"" > outfile
print "    quit" > outfile
close outfile
command="chmod u+x "outfile;system(command);system(outfile)
return
}
```

```
function PLOT2(basefile)    {
#####################################################################
# Plot position of specific spikes
#####################################################################
if(basefile=="") {print"\[ERROR]: set input file first!";return}

FS=" ";split("",tmp),y=0
infile=basefile".spk"
print"\n    PLOT SPIKE POSITIONS"
print"    ~~~~~~~~~~~~~~~~~~~~"
print "    * reading \""infile"\""
FS=" "
while((getline $0 < infile) > 0)                {
                if($2>=0.001)                                   {
                                                                        {
                                                outfile=basefile"_spk"$4".tmp"
                                                tmp[$4]=1
                                                printf("%9s %9s %3s\n",$2,$3,$4) > outfile
                                                                        }
                                                                        }
                                                                        }
close infile; for(x in tmp) {outfile=basefile"_spk"x".tmp";close outfile}

printf("    * executing spike plot of clusters ")
for (x=0;x<=14;x++) if(x in tmp) {y++;printf(x", ")}
print""
z=-1
outfile="plot.tmp"
print "#! \usr\local\bin\gnuplot\n" > outfile
print "set bar 0.05" > outfile
print "set bmargin 3\nset lmargin 3\n" > outfile
print "set xlabel \"x-coordinate\" 0,-2" > outfile
print "set ylabel \"y-coordinate\" -2, 0" > outfile
print "set xtics axis mirror 10" > outfile
print "set ytics axis mirror 10" > outfile
print "set xrange [0:101]" > outfile
print "set yrange [0:101]" > outfile
print "set nogrid" > outfile
print "set nokey" > outfile
print "" > outfile
z=-1
print "    * generating plot "basefile"_spkplot.fig"
print "set term fig color portrait inches size "8.5*(y/2)" "11*(y/2) > outfile
print "set output \""basefile"_spkplot.fig\"" > outfile
print "set multiplot" > outfile
print "set size "(0.5/y)","(0.5/y) > outfile
for (x=0;x<=15;x++) if(x in tmp) {{
        z++; infile=basefile"_spk"x".tmp"
        if(y<=1)  {
                                print"set origin 0,0" > outfile
                                print "set title \""infile"\"" > outfile
                                        }
        if(y>=2)  {
                                print"set origin "z*(1/(y+1.75))",0" > outfile
                                print "set title \""infile"\"" > outfile
                                        }
        print "plot \""infile"\" using 1:2 with dots" > outfile
                                                                }}
print "set nomultiplot\n" > outfile
close outfile
command="chmod u+x "outfile;system(command);system(outfile)
return
}



function PLOT3(basefile,xbintot,ybintot) {
#########################################################
# read means data, convert to proportion of max rate
# sort means for xy bins on scale of 1-10
# assign colours
# generate fig file
#########################################################
FS=" "
xorig=100;yorig=100
lt=0;lw=1;size=250
colour[1]="000000"
colour[2]="000088"
colour[3]="0000ff"
colour[4]="00979d"
```

```
colour[5]="00c668"
colour[6]="00ff00"
colour[7]="caff00"
colour[8]="fff382"
colour[9]="ffffc8"
colour[10]="ffffff"

print"\n   GENERATE XFIG PLOT OF CLUSTER FIRING RATES"
print"   ------------------------------------"
if(basefile=="") {print"\[ERROR]: set input file first!";return}
if(xbintot=="")  {print"\[ERROR]: set x-bins and y-bins first!",return}
print"    * Plot of "basefile" bin in a "xbintot"x"ybintot" matrix."
close "/dev/tty"

outfile=basefile"_rateplot.fig"
print"    * generating xfig file \""outfile"\""
print"#FIG 3.2" > outfile
print"Portrait" > outfile
print"Flush left" > outfile
print"Inches" > outfile
print"Letter" > outfile
print"100.00" > outfile
print"Single" > outfile
print"-2" > outfile
print"1200 2" > outfile
for(z=1;z<=10;z++) print"0 "31+z" #"colour[z] > outfile

infile=basefile".sta"
print"    * reading parameters from \""infile"\""
z=0
while((getline $0 < infile) > 0)   {
                z++
      if(z==1) {xmin=$1;ymin=$3;xmax=$5;ymax=$7;tsamp=$9;tspike=$11;ttime=$13;continue}
      if($1!="") {c=$1;id[c]=$2;cspike[c]=$3;crate[c]=$4}
                        }
close infile

for(origcluster=0;origcluster<=11;origcluster++)              {
if(id[origcluster]<=0) continue

cluster=(id[origcluster]+3)
infile=basefile" bin"
cmax=0

print"    * reading cluster "origcluster" firing rates from \""infile"\""
while((getline $0 < infile) > 0)                                    {
                x=$1;y=$2;rate[x,y]=$cluster
                if(cmax<=$cluster) cmax=$cluster
                                                                                    }
close infile

xmin=xorig+size;ymin=yorig+size
xmax=xorig+((xbintot+1)*size);ymax=yorig+((ybintot+1)*size)
print"6 "xmin" "ymin" "xmax" "ymax > outfile

xbin=ybin=0
modrate=int((rate[x,y]/(cmax+0.001))*10)+1
print"2 2 "lt" "lw" 4 "31+modrate" 0 9 20 0.0000 0 0 -1 0 0 5" > outfile
printf("      ") > outfile
printf(xorig+(xbin*size)" "yorig+(ybin*size)" ") > outfile
printf(xorig+(xbin*size)+size" "yorig+(ybin*size)" ") > outfile
printf(xorig+(xbin*size)+size" "yorig+(ybin*size)+size" ") > outfile
printf(xorig+(xbin*size)" "yorig+(ybin*size)+size" ") > outfile
printf(xorig+(xbin*size)" "yorig+(ybin*size)"\n") > outfile

for(xbin=1;xbin<=xbintot;xbin++) {
for(ybin=1;ybin<=ybintot;ybin++) {

y=ybintot+1-ybin
modrate=int((rate[xbin,ybin]/(cmax+0.001))*10)+1
print"2 2 "lt" "lw" 4 "31+modrate" 0 9 20 0.0000 0 0 -1 0 0 5" > outfile
printf("      ") > outfile
printf(xorig+(xbin*size)" "yorig+(y*size)" ") > outfile
printf(xorig+(xbin*size)+size" "yorig+(y*size)" ") > outfile
printf(xorig+(xbin*size)+size" "yorig+(y*size)+size" ") > outfile
printf(xorig+(xbin*size)" "yorig+(y*size)+size" ") > outfile
printf(xorig+(xbin*size)" "yorig+(y*size)"\n") > outfile
}
}
print"-6" > outfile
cluster=origcluster
printf("4 0 0 0 0 0 14 0.0000 4 0 0 %s %s Cluster %s, %s %c001\n",
                xmin,ymax+(1*size),cluster,basefile,92) > outfile
printf("4 0 0 0 0 0 14 0.0000 4 0 0 %s %s Maxrate = %s Hz%c001\n",
```

```
                xmin,ymax+(2*size),cmax,92) > outfile
printf("4 0 0 0 0 0 14 0.0000 4 0 0 %s %s Meanrate= %s Hz%c001\n",
                xmin,ymax+(3*size),crate[cluster],92) > outfile


xorig+=(xbintot+2)*size
}
for(z=1;z<=10;z++)              {
                print"2 2 "lt" "lw" 4 "31+z" 0 9 20 0.0000 0 0 -1 0 0 5" > outfile
                printf("      ") > outfile
                printf(xmax+size  " "yorig+(z*size)" ") > outfile
                printf(xmax+2*size" "yorig+(z*size)" ") > outfile
                printf(xmax+2*size" "yorig+(z*size)+size" ") > outfile
                printf(xmax+size  " "yorig+(z*size)+size" ") > outfile
                printf(xmax+size  " "yorig+(z*size)"\n") > outfile
                                        }
print"6 "xmax+size" "yorig+(size)" "xmax+2*size" "yorig+(10*size)+size > outfile
for(z=1;z<=10;z++)      {
                printf("4 0 0 0 0 0 14 0.0000 4 0 0 %s %s %2s-%2s%c of max%c001\n",
                                xmax+2.5*size,int(yorig+(z*size)+(0.75*size)),(z-1)*10,z*10,37,92) > outfile
                                        }
close outfile



return
}


function FILE()                 {
#########################################################################
print"\n    SET ORIGINAL FILENAME"
print"    ---------------------"
printf("    * Input filename: "); getline temp < "/dev/tty"
                if(temp=="") {print"     * invalid filename: ABORTED\n\n";return}
                close "/dev/tty"

command="ls -l > file.tmp";system(command)
match(temp,"\\");z=RSTART;if(z<=0)z=length(temp)+1;y=substr(temp,1,z-1)
infile="file.tmp"
status=""
while((getline $0 < "file.tmp") > 0)            {
                if($9==temp) {status=status"A"; print"    * Original datafile found"}
                if($9=="y".sta") {status=status"B"; print"    * Statistics file found"}
                if($9=="y".spk") {status=status"C"; print"    * Spike data compiled"}
                if($9=="y".pos") {status=status"D"; print"    * Position data compiled"}
                if($9=="y".bin") {status=status"E";z="y".bin"
                                while((getline $0 < z) > 0) {xbintot=$1;ybintot=$2}
                                close z
                                print"    * Bin-sort completed: xbins="xbintot", ybins="ybintot
                                        }
                if($9=="y".rot") {status=status"F"; print"    * Rate map rotation performed"}
                                                                        }
close "file.tmp"
if(status=="") {print "    * ERROR - No files found matching "temp;return}
match(status,"E");if(RSTART<=0)xbintot=ybintot=""
origfile=temp
basefile="y
return
}


function BINS()                 {
#########################################################################
print"\n    SET NUMBER OF BINS TO DIVIDE ENVIRONMENT INTO"
print"    ---------------------------------------------"
printf("    * Total x-bins: "); getline x < "/dev/tty"
                if(x=="") {print"     * invalid x-bins: ABORTED\n\n";return}
printf("    * Total y-bins: "); getline y < "/dev/tty"
                if(y=="") {print"     * invalid y-bins: ABORTED\n\n";return}
printf("    * Proceed (y/n)? "); getline GO < "/dev/tty"
        if(GO!="y") {printf("    * ABORTED\n\n"); return}
close "/dev/tty"
z=0;if(x!=xbintot || y!=ybintot) z=1
xbintot=x;ybintot=y
if(z==1) PLACE2(basefile,xbintot,ybintot)
return
}


function ROTATE(basefile)  {
#########################################################################
# Make rotated versions of place field maps
# Input = .bin files (output from PLACE program)...
```

```
#               $1 = xbin
#               $2 = ybin
#               $3 = dwelltime in that bin
#               $4 = firing rate for cluster in that bin
#
# Output on each line...à xbin ybin R  0deg 90deg 180deg 270deg
#####################################################################
print"   MAKE ROTATED VERSIONS OF RATE MAP"
print"   ---------------------------------"
if(basefile=="") {print"   [ERROR]: set input file first!";return}
match(status,"E")
if(RSTART<=0)   {print"   [ERROR]: create rate (.bin) file first!";return}

split("",rate1);split("",rate2);split("",rate3);split("",rate4)
infile=basefile".bin"
outfile=basefile".rot"
print"   * reading min/max bins from "infile
while((getline $0 < infile) > 0)                {xmax=$1;ymax=$2}
close infile
print"   * creating outfile "outfile
while((getline $0 < infile) > 0)                {
                xbin=$1;ybin=$2;z=$4
                if(xbin==0) {print $0 > outfile; continue}
                rate1[xbin,ybin]=z
                rate2[ybin,xmax-(xbin-1)]=z
                rate3[xmax-(xbin-1),ymax-(ybin-1)]=z
                rate4[ymax-(ybin-1),xbin]=z
                                                                        }
close infile
for(x=1;x<=xmax;x++)        {
for(y=1;y<=ymax;y++)        {
                printf("%3s %3s   %7.3f  %7.3f  %7.3f  %7.3f\n",
                x,y,rate1[x,y],rate2[x,y],rate3[x,y],rate4[x,y]) > outfile
                                                }
                                                }
close outfile
status=status"F"
return
}


function SPLIT(basefile)      {
#############################################################################
print"   SPLIT ORIGINAL DATAFILE ("basefile".txt) IN HALF"
print"   ------------------------------------------------"
if(basefile=="") {print"\[ERROR]: set input file first!";return}

FS="."
x=y=z=0
temp="h123456789"
infile=basefile".txt"
while((getline $0 < infile) > 0) {x++}
close infile
outfile=basefile"_a.txt"
print"   * sending 1st half to "outfile
z=0
while((getline $0 < infile) > 0) {
                y++
                if(y>=x/2) {
                                outfile=basefile"_b.txt"
                                if(z==0) {
                                                close outfile
                                                print"   * sending 2nd half to "outfile
                                                 }
                                z=1
                                  }
                print $0 > outfile
                                                        }
close infile;close outfile
return
}


function CLUSTERS(basefile)                {
#############################################################################
print"\n   * MODIFY CLUSTERS IN "origfile
print"   * ------------------------------"
if(basefile=="") {print"\[ERROR]: set input file first!";return}

printf("   * Replace cluster#: "); getline x < "/dev/tty"
                if(x=="") {print"   * invalid cluster: ABORTED\n\n";return}
printf("   *           with: "); getline y < "/dev/tty"
                if(y=="") {print"   * Remove cluster #"x}
```

```
printf("    * THIS IS NON-REVERSIBLE! PROCEDE? ");getline z < "/dev/tty"
 if(z!="y") {print"    * ABORTED\n\n";return}
close "/dev/tty"
z=0
FS=","
infile=origfile
outfile="file.tmp"
while((getline $0 < infile) > 0)                {
                if($1!="S" && $1!="E") {print $0 > outfile;continue}
                if($3!=x) {print $0 > outfile;continue}
                w++
                if(y=="") continue
                $3=y
                for(z=1;z<=NF-1;z++) printf($z",") > outfile
                print $z  > outfile
                                                                        }
close infile;close outfile
command="mv "outfile" "infile;system(command)
print "    * "w" replacements made"
return
}

function OPTIONS()          {
##############################################################################
print ""
print"    1: read data file"
print"    2: calculate bin firing rates"
print""
print"    p1: plot all position samples"
print"    p2: plot spike positions by cluster"
print"    p3: generate rate map"
print""
print"    o: options"
print"    f: set input filename"
print"    c: modify clusters in original file"
print"    b: set x-bins & y-bins"
print"    ls: directory"
print"    r: create rotated versions of rate map"
print"    s: split original data file in two"
print"    q: quit"
}


function QUIT()             {
##############################################################################
print"\nQUIT PROGRAM\n",exit
}
function LS()               {
##############################################################################
print"";command=$0;system(command)
}


rm -f *_pos* tmp
rm -f *_spk* tmp
rm -f file.tmp
```

Appendix B
Correlation Matrices

Subject 9

August 11

	D0	D90	D180	D270
D0	1.0000	-.0311	-.1376	-.0311
D90	-.0311	1.0000	-.0311	-.1376
D180	-.1376	-.0311	1.0000	-.0311
D270	-.0311	-.1376	-.0311	1.0000
B	.5011**	-.0302	-.1719	.0195
C	.6641**	-.0446	-.2004*	-.0374
E	.6749**	.0368	-.3064**	-.0614
F	.2829**	-.0897	-.1136	-.0167
G	.5879**	.1147	-.1316	-.1879
H	.6806**	.0871	-.2486*	-.1914
I	.4305**	-.0122	-.2533*	-.1659
J	.0827	.4878**	-.0574	-.2889**
K	-.1815	-.0469	.5356**	.0298
L	.0683	-.2852**	-.0602	.3143**

AUGUST 13

	D0	D90	D180	D270
D0	1.0000	.0803	-.0421	.0803
D90	.0803	1.0000	.0803	-.0421
D180	-.0421	.0803	1.0000	.0803
D270	.0803	-.0421	.0803	1.0000
B	-.0873	-.0959	.0760	.3583**
C	-.1167	.0659	.3250**	-.0500
E	.5186**	-.0642	-.1704	-.0574

AUGUST 14

	D0	D90	D180	D270
D0	1.0000	.0091	.0333	.0091
D90	.0091	1.0000	.0091	.0333
D180	.0333	.0091	1.0000	.0091
D270	.0091	.0333	.0091	1.0000
B	.0152	.0322	-.0914	.0103
C	-.0963	-.0497	.1207	.0172

Subject 13

August 20 part 1

	D0	D90	D180	D270	B	C	D	E	F
D0	1.0000	-.2226*	-.1582	-.2226*	.6234**	.5832**	.4642**	.5065**	.6026**
D90	-.2226*	1.0000	-.2226*	-.1582	-.1445	-.2648**	.0955	-.1571	-.0613
D180	-.1582	-.2226*	1.0000	-.2226*	-.2543*	-.1838	-.1987*	-.2976**	-.3733**
D270	-.2226*	-.1582	-.2226*	1.0000	-.1372	-.1700	-.2398*	-.1535	-.1658
B	.6234**	-.1445	-.2543*	-.1372	1.0000	.6124**	.5080**	.7090**	.6610**
C	.5832**	-.2648**	-.1838	-.1700	.6124**	1.0000	.4658**	.6100**	.5985**
D	.4642**	.0955	-.1987*	-.2398*	.5080**	.4658**	1.0000	.5280**	.5114**
E	.5065**	-.1571	-.2976**	-.1535	.7090**	.6100**	.5280**	1.0000	.7935**
F	.6026**	-.0613	-.3733**	-.1658	.6610**	.5985**	.5114**	.7935**	1.0000

August 20 part 2

	D0	D90	D180	D270	H	I	J
D0	1.0000	-.1244	-.1576	-.1244	.3410**	.1415	.2009*

D90	-.1244	1.0000	-.1244	-.1576	-.0328	.3406**	-.0227
D180	-.1576	-.1244	1.0000	-.1244	-.1043	-.1985*	-.1591
D270	-.1244	-.1576	-.1244	1.0000	.0372	-.1700	.1071
H	.3410**	-.0328	-.1043	.0372	1.0000	.1052	-.1471
I	.1415	.3406**	-.1985*	-.1700	.1052	1.0000	.1089
J	.2009*	-.0227	-.1591	.1071	-.1471	.1089	1.0000

Subject 15

JUN5

	D0	D90	D180	D270
D0	1.0000	-.0367	-.0339	-.0367
D90	-.0367	1.0000	-.0367	-.0339
D180	-.0339	-.0367	1.0000	-.0367
D270	-.0367	-.0339	-.0367	1.0000
DAT_B	.7232**	.0282	-.1334	.0683
DAT_C	-.0292	-.2157*	.1160	.6574**
DAT_D	.2713**	-.1295	-.1849	-.1290
DAT_E	.6780**	-.0351	-.1150	-.0907
DAT_F	.7769**	-.0990	-.1763	-.0614
DAT_G	.5024**	-.0915	-.1921	.0377
DAT_H	.6670**	-.0358	-.1582	-.0325

JUNE 30

	D0	D90	D180	D270
D0	1.0000	-.1245	-.1803	-.1245
D90	-.1245	1.0000	-.1245	-.1803
D180	-.1803	-.1245	1.0000	-.1245
D270	-.1245	-.1803	-.1245	1.0000
DAT_B	.8272**	-.0674	-.1979*	-.1425
DAT_C	.7927**	-.0652	-.2214*	-.0925
DAT_D	.6899**	-.0824	-.2266*	-.0028
DAT_E	.0643	-.2290*	-.1389	.7811**
DAT_F	-.2240*	-.1372	.7518**	.1388
DAT_G	-.0715	-.1931	-.1324	.7389**
DAT_H	.8893**	-.0280	-.2064*	-.1263

JULY 1

	D0	D90	D180	D270
D0	1.0000	-.0771	-.3385**	-.0771
D90	-.0771	1.0000	-.0771	-.3385**
D180	-.3385**	-.0771	1.0000	-.0771
D270	-.0771	-.3385**	-.0771	1.0000
DAT_B	.8722**	-.0298	-.3382**	-.1599
DAT_C	.7734**	.0811	-.3097**	-.2254*
DAT_D	-.3044**	-.1990*	.7950**	.0514

JULY 2

	D0	D90	D180	D270
D0	1.0000	-.0778	-.3139**	-.0778
D90	-.0778	1.0000	-.0778	-.3139**
D180	-.3139**	-.0778	1.0000	-.0778
D270	-.0778	-.3139**	-.0778	1.0000
DAT_B	.8873**	-.0954	-.3186**	-.0247

JULY 3

	D0	D90	D180	D270
D0	1.0000	-.1037	-.1425	-.1037
D90	-.1037	1.0000	-.1037	-.1425
D180	-.1425	-.1037	1.0000	-.1037
D270	-.1037	-.1425	-.1037	1.0000
DAT_B	-.0969	.6287**	-.0965	-.0164

DAT_C	.7136**	-.1045	-.1109	-.1279
DAT_D	-.0760	-.0902	-.0853	.6699**
DAT_E	-.1368	-.0588	.7738**	-.1032
DAT_F	.7789**	-.1213	-.1137	-.0759
DAT_G	.7412**	-.1599	-.1463	-.0226
DAT_H	.0159	-.1699	-.0327	.7064**

JULY 6

	D0	D90	D180	D270
D0	1.0000	.0621	-.2460*	.0621
D90	.0621	1.0000	.0621	-.2460*
D180	-.2460*	.0621	1.0000	.0621
D270	.0621	-.2460*	.0621	1.0000
DAT_B	-.0575	-.1803	.0856	.6635**
DAT_C	.7799**	-.0453	-.2017*	-.0199
DAT_D	-.1254	-.2062*	.0238	.6277**

JULY 7

	D0	D90	D180	D270
D0	1.0000	.0927	-.1205	.0927
D90	.0927	1.0000	.0927	-.1205
D180	-.1205	.0927	1.0000	.0927
D270	.0927	-.1205	.0927	1.0000
DAT_B	-.0293	-.0511	.2865**	-.0406
DAT_C	.4376**	.0966	-.0703	.0173
DAT_D	-.0401	.4670**	-.0446	-.1092
DAT_E	.4608**	.1136	-.0986	.2338*

JULY 8

	D0	D90	D180	D270
D0	1.0000	-.1325	-.1483	-.1325
D90	-.1325	1.0000	-.1325	-.1483
D180	-.1483	-.1325	1.0000	-.1325
D270	-.1325	-.1483	-.1325	1.0000
DAT_B	.5910**	-.0214	-.1108	-.1189
DAT_C	.6818**	-.1386	-.0280	-.1117
DAT_D	.3790**	-.0496	-.0736	-.0850

* - Signif. LE .05 ** - Signif. LE .01 (2-tailed) . " printed if a coefficient cannot be computed